MYCORRHIZAL STATUS AND AMF COMMUNITY STRUCTURE OF FRUIT CROPS FROM LOW-INPUT CROPPING SYSTEM IN SHOWA ROBIT, ETHIOPIA

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

ABSTRACT: Arbuscular mycorrhizal fungi (AMF) association of *Mangifera indica* (mango), *Musa acuminate* (banana), *Carica papaya* (papaya), *Citrus limon* (lemon), *Persea americana* (avocado), and *Psidium guajava* (guava) was investigated from a lowland area of Showa Robit. Percentage of root colonization, spore abundance, species richness and diversity were examined. The result showed that fruit crops fell into higher spore density group of (7.2- 8.8 spores g^{-1} of soil) and low spore density group (3.7-5.3 spores g^{-1} of soil). Accordingly, spore density from mango, avocado, banana and lemon belongs to the high density spore group; whereas, spore from papaya and guava fell into the low spore density group at P<0.05. The AM colonization also showed that mango and lemon have high mycorrhization (71.7%), and guava have a low mycorrhization of 27.3%. A total of 32 morphospecies belonging to 12 genera were characterized from all the fruit crops. The highest AMF species richness was for mango (18 species) followed by banana (16 species) and guava (14 species). The species *Claroideoglomus claroideum* and *Glomus aggregatum* were the dominant species ("generalists") among the fruit trees. A total of 13 AMF species were detected in only one of the tested fruits, out of which four species were recovered from mango indicating that the crops are selective to specific mycorrhizal fungi.

Key words/phrases: Arbuscular mycorrhizal fungi, *Claroideoglomus*, Fruit crops, *Glomus*, Root colonization.

INTRODUCTION

Banana, papaya, mango, guava, orange, avocado, and lemon are important fruit crops for both domestic consumption and income generation for farmers, export markets and industrial processing in Ethiopia. According to CSA (2012), the total area under fruits in 2011/2012 was about 61,973 hectares which was 0.46% of the total land area under cultivation in the country.

Although the land area for fruit is lower in comparison to cereals, they are mostly produced in low-input intercropping and agroforestry systems and are good sources of cash for many small-scale farmers. The major

¹ Department of Microbial, Cellular and Molecular Biology, College of Natural Sciences, Addis Ababa University, P.O. Box 1176, Addis Ababa, Ethiopia. E-mail[: zebelay@gmail.com;](mailto:zebelay@gmail.com) asefafasil2013@gmail.com

² Natural Resources Institute, Finland (Luke), Laukaa, Finland. E-mail[: mauritz.vestberg@mtt.fi](mailto:mauritz.vestberg@mtt.fi)

^{*} Author to whom all correspondence should be addressed

constraints for less area coverage of these crops is low productivity due to low soil fertility, low agricultural inputs and increased pressure of pests and diseases (Alemayehu Seyoum *et al*., 2011; Gaidashova *et al*., 2012). Experiences from elsewhere indicate that these problems can be tackled using integrated soil fertility (ISFM) and integrated pest management (IPM) packaged in cultural, chemical and biological systems in order to enhance production of crops in general, and that of fruits in particular (Lovato *et al*.,1995).

Arbuscular mycorrhizal fungi (AMF) are one of the biological components that can contribute to enhancement of soil fertility and nutrient uptake of plants so as to boost production of crops. They are soil organisms known to penetrate deep into the cortical cells of roots and extend their extraradical hyphae outside the root so as to serve as extensions of plant roots system. AMF increase the absorptive surface area of the root system for nutrient and water uptake particularly phosphorus (P) (Smith and Read, 2008), nitrogen (N) and other immobile nutrients (Sawers *et al*., 2008). Many studies showed that AMF associations with plants increase shoot and root biomass as well as plant tissue nutrient concentrations, enhance tolerance for drought conditions (Augé, 2001), and increase resistance to soil-borne pathogens (Wehner *et al*., 2010).

Fruit crops are one of the vascular plants that are associated with arbuscular mycorrhizal fungi (AMF) (Khade and Rodrigues, 2009). These crops are known to harbour diverse group of AMF spores and that cover up to 50% of the plant roots (mycorrhization) depending upon the contents of P and other nutrients in the soil (Gaidashova *et al*., 2012; Abdellhalim *et al*., 2013). There are also suggestions that different management and land use systems influence the density and diversity of mycorrhiza in the rhizosphere of fruit crops (Alarcón *et al*., 2012).

Previous studies showed that there were large differences in the mycorrhizal dependency values among tropical fruit tree species. Jaizme-Vega and Azcón (1995), under green house and field conditions in the Canary Islands (Spain), reported the mycorrhizal dependency for avocado (30.7%), banana (105%) and papaya (75.8%) after inoculated with four different AM fungi species. Under controlled conditions, several citrus cultivars under three nutrient regimes have shown 82-91% of mycorrhizal dependency (Menge *et al*., 1978).

Reports in Ethiopia revealed that species composition and community structure of AMF vary depending upon the type of plants in acacia woodland (Yonas Yohannes and Fassil Assefa, 2007) and diversity of shade trees in coffee agroforestry system (Diriba Muleta *et al*., 2008; Tadesse Chanie and Fassil Assefa, 2013). However, studies on the role of AMF in fruit crops are very limited.

Recently, the urgent need for low-input plant production and reduction of chemical inputs in the sector necessitated alternative approaches that involve soil microorganisms. These alternatives should take into consideration the role of AMF a beneficial soil organism to potentially protect crops from pests and diseases, and boost production.

The objectives of this study were to evaluate AMF root colonization, spore density, and AMF community structure of different fruit crops under lowinput fruit production in Showa Robit, Ethiopia.

MATERIALS AND METHODS

Study site

The study was undertaken in Showa Robit, Amhara Regional State, Ethiopia, located at 09° 57′ N, and 039° 51′ E, and an altitude of 1305 m above sea level. The area is a lowland or Erteb Kola (sub-moist warm) with an average annual maximum temperature of 32°C, minimum temperature of 16°C and precipitation of 968 mm (NMA, 2010). The physico-chemical properties of the soil samples are given in Table 1.

Table 1. Physical and chemical properties of soil samples from rhizosphere soil of six fruit plants.

P: available phosphorus; T.N: total nitrogen; O.C: organic carbon; ppm: parts per million

The area is characterized by agroforestry practices such as agrisilvicultural (crops and shrubs/trees) and agropastoral systems (crops and pastures/animals and trees). In the fruit cropping area (FC), mainly fruits, vegetables and garden cash crops were grown in an intercropping system with banana, papaya, mango, lemon, avocado, tomato and coffee. The dominant management practice of the area is low-input system, in which manures, compost and crop residues are applied.

Collection of soil and root samples

The sampling was done in November, 2011 during the dry season of the year. Six different fruit crops were selected for the survey. They were mango, banana, papaya, lemon, avocado and guava. Voucher specimens of the crops were brought to the National Herbarium for identification /verification.

The samples were taken from 10 $m \times 10$ m transects in three replicates of sampling locations from approximately 1 sqkm of the agricultural field. Three replicates of each fruit crop species were randomly selected in each sampling locations. From each sampling location, 500 g of rhizosphere soil samples of each fruit crop species were taken from a depth of 0-30 cm and subsequently pooled into one composite sample per location.

A total of 18 samples, 3×6 from the fruit crop field were collected. The samples were collected in alcohol sterilized plastic containers, air dried and stored at room temperature for further analysis. Fine root samples from each individual crop were also collected and stored in 50% alcohol at 4°C for determination of root colonization by AM fungi (Brundrett *et al*., 1996).

Spore extraction and identification

Soil samples were air-dried before extraction, followed by counting and identification of AM fungal spores. AMF spores from the soil samples were extracted by the wet sieving and decanting method (Gerdemann and Nicolson, 1963), followed by centrifugation in water in 50% sucrose solution (Brundrett *et al*., 1996). 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 μ m sieves were counted and observed by using a dissecting microscope. The spores were then 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. The AMF spores present were morphologically identified at the Department of Microbial, Cellular and Molecular Biology, Addis Ababa University, Ethiopia, and Natural Resources Institute Finland (Luke), Laukaa, Finland. Identification and classification were based on a current species description 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/.](http://www.lrz.de/~schuessler/amphylo/)

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.

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. With the exception of the HCl treatment, samples were drained and washed thoroughly with distilled water at the end of every action. Fungal colonization was quantified using the magnified intersection method of McGonigle *et al*. (1990) under a compound-light microscope (OLYMPUS-BX51) at a magnification of 200 X. Accordingly, 150 intersections were observed for each sample. The presence of arbuscular mycorrhizal hyphae, vesicles and arbuscules were recorded.

Determination of spore density and diversity of AMF

Spore density (SD) was expressed as the number of AMF spores g^{-1} soil. Species richness (S) was measured as the total number of morphospecies. The Shannon-Wiener index (H') of diversity was calculated using the formula: H' = $-\sum$ ((n_i /n) ln (n_i/n)) where: n_i = number of individuals of species i and $n =$ number of all individuals of all species. The dominance index (D) was calculated using the formula $D = \sum (n_i/n)^2$; Evenness (E) was calculated by dividing Shannon-Wiener diversity value by the logarithm of the species richness. These analyses were conducted using the software PAST3 (ver. 3.0).

Isolation frequency (IF) was calculated as (the number of samples in which a given species was isolated/ the total number of samples) \times 100%. Relative abundance of spores (RA) was calculated as (the number of spores in a given species/total number of spores) \times 100%. Dominant AMF species were determined by the importance value (IV) based on IF and RA and was

calculated as IV = $(\text{IF} + \text{RA})/2$. An IV $\geq 50\%$ indicates that a genus or species is dominant; $10\% < IV < 50\%$ applies to common genera or species; an IV $\leq 10\%$ indicates that a genus or species is rare (Chen *et al.*, 2012).

Statistical analysis

Spore abundance data were $log(x)$ transformed and the proportion of root colonization were square root $[(x+0.5)^{1/2}]$ and arcsine (the inverse sine of the square root of the proportion) transformed prior to analysis to meet assumptions of ANOVA such as normality and homogeneity of variance, but values were expressed as number of spores g^{-1} soil and percentage of root colonization, respectively.

Analysis of variance (ANOVA) and correlation analysis were carried out with the SPSS software package (version 21.0). Significance differences in AM fungal spore abundance and percentage of root colonization between the plants were tested using Fisher's least significant difference (LSD) at p<0.05 after one-way analysis of variance (ANOVA). The relationship between AMF parameters and soil chemical properties (pH, OC, available P, and TN) was determined by Pearson's correlation analysis.

RESULTS AND DISCUSSION

Spore abundance

Spore abundance (density) from the soils of the different fruit crops showed variation between the higher spore density group $(7.2{\text -}8.8 \text{ spores } g^{-1})$ and low spore density group $(3.7-5.3 \text{ spores } g^{-1} \text{ soil})$. Accordingly, mango, avocado, banana, and lemon were characterized by high density spore group whereas, papaya, and guava fell into the low spore density group at $P<0.05$ (Table 2).

The pattern of spore densities of the fruits in this study was also compared to other studies in different countries (Table 2). Although the spore density from the rhizosphere of mango was almost twice and three times more abundant than the ones recorded from Central Sudan $(4.0 \text{ spores } g^{-1} \text{ of soil})$ (Abdellhalim *et al.*, 2013) and Bangladesh (2.8 spores g^{-1} of soil) (Khanam, 2007), the spore density under banana was much less than the spore number recorded from the Sudan (Abdellhalim *et al*., 2013). However, the spore density under lemon was similar to the finding of Abdellhalim *et al*. (2013), but ten times higher than spore density recorded from Bangladesh (Khanam, 2007) (Table 2).

Fruit crops	Ethiopia (Showa Robit)	Sudan (Abdellhalim et al., 2013)	Bangladesh (Khanam, 2007)	Brazil (Trindade et al., 2006)
Mango	$8.8 \pm 0.9c$	4	2.8	$\overline{}$
Banana	7.3 ± 1 bc	12	۰	$\overline{}$
Papaya	$3.7 \pm 0.1^{\circ}$	-	۰	7.8
Lemon	7.2 ± 0.6 bc		0.7	$\overline{}$
Avocado	$7.8 \pm 1c$		۰	$\overline{}$
Guava	$5.3 \pm 0.6ab$	۰	3.2	$\overline{}$

Table 2. AMF spore abundance $(g^{-1} \text{ soil})$ of different fruit crops from different parts of the world.

Data are reported as averages and standard errors for three replicates per plant type. Values followed by different letters denote significant differences among fruit crops according to Fisher's LSD test at the 5% level after a oneway ANOVA.

The spore density recorded from soil under guava $(5.3 \text{ spores } g^{-1} \text{ of soil})$ was higher than the spore density recorded from Bangladesh (3.2 spores g^{-1}) of soil). Even if lemon showed the same number of spore with the Sudan, the spore density of papaya in this study $(3.7 \text{ sports g}^{-1}$ of soil) was more than twice lower than the spore density recorded from Brazil (7.8 spores g^{-1}) of soil) (Trinidade *et al*., 2006).

The variation revealed in terms of spore density among different fruit crops could be attributed to host preference (Bever *et al*., 1996; Mathimaran *et al*. (2007), and the inconsistency in spore abundance among the same fruit crops in different areas might be related to the difference in their responses to specific environmental factors (Muthukumar and Udaiyan, 2002). Khanam (2007) counted more than 10 times higher spore densities from the same fruits and sampling sites in Bangladesh within three years.

AMF root colonization

The roots of the different fruits were colonized by all AM fungal structures, i.e., arbuscules, vesicles and hyphae with different pattern of percentage of colonization (Fig. 1, Table 3). The mean percentage of hyphal colonization across all fruit crops was between 27.3% and 71.7%. Hyphal colonization was significantly higher in mango (71.7%), lemon (71%) and avocado roots (66.3%) compared to papaya (54.7%), banana (46%) and guava (27.3%). The arbuscule and vesicle colonization of the different crops was 1.3-10% and 0.3-20%, respectively.

The data also showed that hyphal colonization in banana, lemon and avocado almost concurred with colonization of arbuscular and vesicular structures, except in mango. This was contrary to the report of Khanam (2007), where no vesicles and arbuscules were detected from the roots of lemon, mango, and guava from horticultural farm of Bangladesh Agricultural Research Institute. The percentage hyphal colonization recorded in this study was higher than the ones recorded for mango (45%), banana (40%), and lemon (30%) in White Nile State, Central Sudan (Abdellhalim *et al*., 2013), and the percentage colonization of the roots of mango (30%), lemon (23%) but similar with root colonization on guava (27%) from horticultural farm in Bangladesh (Khanam, 2007).

Fig. 1. Arbuscular mycorrhizal colonization in the roots of fruit crops: (a) network of extraradical mycelium (EM); (b) a typical arbuscule (A) in a cortical cell and (c) & (d) vesicle (V) and intracellular mycelium (IM) in root cell.

Table 3. Percentage of AM fungal root colonization in six fruit crops at Showa Robit, Ethiopia.

Data are reported as averages and standard errors for three replicates per land use types. Values followed by different letters denote significant differences among fruit crops according to Fisher's LSD test at the 5% level after a one-way ANOVA.

In general, the high-mycorrhization fruits; mango and lemon displayed more than twice the root colonization of the same fruits reported from that of Sudan (Abdellhalim *et al*., 2013) and Bangladesh (Khanam, 2007). It is interesting to note that the pattern of colonization on papaya (54.7%) was similar to a study in India (50%) (Khade and Rodrigues, 2009) but higher than a study in Brazil (31%) (Trinidade *et al*., 2006). In this study guava (27.3%) also had a similar pattern of hyphal colonization reported from Bangladesh (26.7%) (Khanam, 2007). The significant variation observed in the colonization of AM fungi among different fruit plants and within the same species might be due to differences in root structure, climatic and soil factors (Khade and Rodrigues, 2009; Gaidashova *et al*., 2012) and AMF diversity and species composition (Jansa *et al*., 2007).

In the present study, slight positive correlation $(r=0.56)$ was observed between AMF root colonization and spore density of AM fungi. This is because the two parameters are influenced by many biotic and abiotic factors such as the type of fungal species, plant host and soil nutrients (Stutz and Morton, 1996). The high mycorrhization of fruits indicates that the fruits, in general, induce high levels of infective propagules in the rhizosphere soil. It has been reported that the numbers of infective propagules are positively correlated with root colonization levels (Azcón-Aguilar *et al*., 2003).

The relationships between the distribution of AMF and soil chemical properties were not significant except that the organic carbon was positively correlated with the percentage of root colonization by arbuscules and vesicles ($r=0.95$, $P<0.01$; $r=0.89$, $P<0.05$, respectively) (data not shown). A similar trend was reported from Brazil by Trindade *et al*. (2006), that showed a positive correlation between AM colonization and organic carbon in papaya plantations $(r=0.32, P<0.01)$.

AMF species richness and diversity

AMF species richness was comparatively variable between the fruit trees. The highest AMF species richness was in mango (18), banana (16), guava (14) and papaya, lemon and avocado (12) (Fig. 2). The number of AM fungal species isolated from banana and lemon was slightly higher than previous investigations from White Nile State, Central Sudan for banana (14) and for lemon (10) but similar for mango (18) (Abdellhalim *et al*., 2013). This is contrary to the detection of only 1 AMF species from banana and 2 species from lemon grown in the different land use systems of Mexico (Alarcón *et al*., 2012).

Fig. 2. AMF genera and species richness in the six species of fruit crops.

However, the AMF species diversity from avocado and papaya in this study was 2-3 times lower than the AMF morphotypes isolated from the rhizosphere soil of avocado (36) in Mexico (Alarcón *et al*., 2012) and papaya (24) from Brazil (Trindade *et al*., 2006). On the other hand, the diversity of AMF from papaya (12) is similar to the number of 13 morphotypes identified from India (Khade and Rodrigues, 2009). It is interesting to note that although guava was characterized by low spore

density and low root colonization, it harboured diverse species of AMF (14 morphospecies) compared to the fruits with higher spore numbers and high mycorrhization such as lemon and avocado. In this study, the number of genera identified from mango, banana, and lemon was similar to those reported from Sudan (Abdellhalim *et al*., 2007).

A total of 32 species representing 12 genera were detected from field soil samples under the rhizosphere of the fruit crops (Fig. 2 and Fig. 3). The dominant genera were *Acaluspora*, *Filiniformis* and *Glomus* which were diversified into 8 species, 6 species, and 6 species of AMF, respectively. They were followed by the genera *Clarioideoglomus* and *Gigaspora* with 3 and 2 representative species, respectively. These genera represented almost 80% of the species recovered from the fruit crops.

In general, the data showed that only 43% of the species were found in the rhizosphere of the half and more of the fruit crops (Table 4). The AMF species community distribution based on important value (IVs) showed that (2 species) *Claroideoglomus claroideum* and *Glomus aggregatum* were distributed in almost all fruits and categorized as dominant species with IVs of 53-60 (Chen *et al*., 2012).

The dominant AMF species detected among the fruit trees appear to be generalist fungi, because they were detected in roots of a wide range of plant species (Stutz *et al.*, 2000; Zhao and Zhao, 2007). Likewise, 17 species were categorized into the "common group" represented by the high IV representative of AMF; with *Claroideoglomus etunicatum*, *Cl. luteum*, *Funneliformis mosseae*, *F*. *caledonium* and *Glomus microcarpum* that were found in all but one fruit crop (IV 42-46) (Table 4). It is interesting to note that 41% of the species recovered from fruit crops were classified in the "rare category" according to the definition of Chen *et al*. (2012). These "rare" AMF species were clustered into one or the other fruit crops indicating that they have strong affiliation for them. Consequently, mango fruit harboured the maximum number of four species that were not found elsewhere whereas, lemon harboured three species, and two species were distributed each in banana and guava. Avocado and papaya harboured one species each that did not occur in any of the other fruits (Table 4).

Table 4. List, isolation frequency (IF), relative abundance (RA) and important values (IVs) of AMF species recovered from the rhizosphere of fruit crops at Showa Robit.

Ethiop. J. Biol. Sci., **13(2): 99-116, 2014** 111

Table 4 continued

It is interesting to note that although *Acaulospora* was represented by the highest number of species (8 species), the distribution was limited to specific fruits as opposed to the genus *Clareoideoglomus* that was represented by a few species and yet was distributed across the majority of the fruits. Most species from *Acaulospora* together with two species from the genus *Gigaspora* were categorized into the "rare" cluster (Table 4).

On the contrary, the genera *Septoglomus*, *Pacispora*, *Entrophospora*, *Diversipora* and *Rhizophagus* were represented by single species; they were categorized into a commonly occurring group. Moreover, these genera were distributed in more than half of the fruits tested indicating that they were relatively widely distributed amidst their limitation in the number of species they contained. In all cases the generalist *Glomus*, *Funniliformis*, *Clarioideoglomus* were identified from these fruits. However, the genera *Rhizophagus*, *Gigaspora* and *Racocerta* were missing from these fruit crops from the Sudan study (Abdellhalim *et al*., 2013), whereas the genera *Ambispora* and *Archeospora* were not detected in this study.

In the present study, the diversity of AMF communities based on the Shannon-Wiener diversity index (Table 5) was the highest in banana $(H'=2.57)$ and the lowest in papaya $(H'=2.02)$. AMF species evenness ranged between 0.82 and 0.63 and the lowest evenness distribution was found in mango and papaya, the highest in banana. The species dominance (D) was the highest in papaya ($D=0.18$) and the lowest in banana ($D=0.09$).

Table 5. Diversity indices of AMF community in six fruit crops.

Fig. 3. Some AMF species identified from rhizosphere soil samples of fruit plants in Ethiopia. All photos are from slides made in PVLG. a) *Septoglomus constrictum*, b) *Acaulospora kentinesis*, c) *Funneliformis mosseae*, d) *Cl*. *luteum*, e) *F*. *caledonium*, f) *Paraglomus occultum*, g) *Acaulospora scrobiculata,* h) *Glomus aggregatum*, i) *Glomus* sp.2, j) *Glomus* sp.1, k) *Ac*. *denticulata*, l) *Gl*. *microaggregatum*.

CONCLUSION AND RECOMMENDATION

In the present study, the association of AM fungi with six fruit tree species was evaluated in low-input cropping systems of Showa Robit. The spore abundance and the species diversity of AMF identified from the fruit crops were relatively large. Highest percentage of AMF root colonization and spore density was recorded from mango whereas the lowest was from papaya and guava.

Generally, AMF species richness, root colonization and spore density recorded in the fruit crops were comparatively variable between the fruit crops which emphasize the fact that, the variation might be associated with host plant species and edaphic factors. However, further steps need to be undertaken to study the functional diversity of AM fungi associated with the roots of these plants and to determine their relative contribution to different mycorrhizal functions.

ACKNOWLEDGEMENTS

We thank the School of Graduate Studies, Addis Ababa University for providing financial assistance.

REFERENCES

- Abdellhalim, T.S., Finckh, M.R., Babiker, A.G. and Oehl, F. (2013). Species composition and diversity of arbuscular mycorrhizal fungi in White Nile state, Central Sudan. *Arch. Ag. Soil Sci*. **60**: 377–391.
- Alarcón, A., Hernández-Cuevas, L.V., Ferrera-Cerrato, R. and Franco-Ramírez, A. (2012). Diversity and agricultural applications of arbuscular mycorrhizal fungi in Mexico. *J. Biofertil. Biopestici.* **3**:1.
- Alemayehu Seyoum, Dorosh, P. and Sinafikeh Asrat (2011). Crop Production in Ethiopia: Regional Patterns and Trends. ESSP II Working Paper 16.
- Augé, R.M. (2001). Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. *Mycorrhiza* **11**: 3–42.
- Azcón-Aguilar, C., Palenzuela, J., Roldán, A., Bautista, S., Vallejo, R. and Barea, J.M. (2003). Analysis of the mycorrhizal potential in the rhizosphere of representative plant species from desertification-threatened Mediterranean shrublands. *Appl. Soil Ecol*. **22**: 29–37.
- Bever, J.D., Morton, J.B., Antonovics, J. and Schultz, P.A. (1996). Host-dependent sporulation and species diversity of arbuscular mycorrhizal fungi in mown grassland. *J. Ecol*. **84**: 71–82.
- Brundrett, M., Bougher, N., Dell, B., Grove, T. and Malajczuk, N. (1996). Working with mycorrhizas in forestry and agriculture. ACIAR Monograph 32. Australian Centre for International Agricultural Research, Canberra.
- CSA (2012). Central Statistics Agency Agricultural Sample Survey. Report on area and production of major crops. Statistical bulletin, V. 1: 532.
- Chen, K., Liu, W., Guo, S., Liu, R. and Li, M. (2012). Diversity of arbuscular mycorrhizal fungi in continuous cropping soils used for pepper production. *Afr. J. Microbiol. Res*. **6**: 2469–2474.
- Diriba Muleta, Fassil Assefa, Sileshi Nemomissa and Granhall, U. (2008). Distribution of arbuscular mycorrhizal fungi spores in soil of southwestern Ethiopia. *Biol. Fert. Soils*. **44**: 653–659.
- [Gaidashova,](http://www.sciencedirect.com/science/article/pii/S0167880912000199) S., [Nsabimana,](http://www.sciencedirect.com/science/article/pii/S0167880912000199) A., [Karamura,](http://www.sciencedirect.com/science/article/pii/S0167880912000199) D., [Asten,](http://www.sciencedirect.com/science/article/pii/S0167880912000199) P. and [Declerck,](http://www.sciencedirect.com/science/article/pii/S0167880912000199) S. (2012). Mycorrhizal colonization of major banana genotypes in six East African

environments. *[Agr. Ecosyst. Environ.](http://www.sciencedirect.com/science/journal/01678809)* **[157](http://www.sciencedirect.com/science/journal/01678809/157/supp/C)**: 40–46.

- Gerdemann, J.W. and Nicolson, T.H. (1963). Spores of mycorrhizal Endogone species extracted from soil by wet sieving and decanting. *T. Brit. Mycol. Soc*. **46**: 235– 244.
- Jaizme-Vega, M.C. and Azcón, R. (1995). Responses of some tropical and subtropical cultures to endomycorrhizal fungi. *Mycorrhiza* **5**: 213–217.
- Jansa, J., Smith, J.F. and Smith, S.E. (2007). Are there benefits of simultaneous root colonization by different arbuscular mycorrhizal fungi? *New Phytol*. **177**: 779– 789.
- Khade, S.W. and Rodrigues, B.F. (2009). Arbuscular mycorrhizal fungi associated with varieties of *Carica papaya* L. in tropical agro-based ecosystem of Goa, India. *Trop. Subtrop. Agroecosyst*. **10**: 369–381.
- Khanam, D. (2007). Assessment of arbuscular mycorrhizal association in some fruit plants in Bangladesh. *Bangladesh J. Microbiol*. **24**: 34–37.
- Lovato, P.E., Schuepp, H., Trouvelot, A. and Gianinazzi, S. (1995). Application of arbuscular mycorrhizal fungi (AMF) in orchard and ornamental plants. In: **Mycorrhiza: Structure, Function, Molecular Biology and Biotechnology,** pp. 443–467 (Varma, A. and Hock, B., eds.). Springer Berlin, Heidelberg.
- Mathimaran, N., Ruh, R., Jama, B., Verchot, L., Frossard, E. and Jansa, J. (2007). Impact of agricultural management on arbuscular mycorrhizal fungal communities in Kenyan ferralsol. *Agr. Ecosyst. Environ*. **119**: 22–32.
- McGonigle, T.P., Miller, M.H., Evans, D.G., Fairchild, G.L. and Swan, J.A. (1990). A new method which gives an objective measure of colonization of roots by vesiculararbuscular mycorrhizal fungi. *New Phytol*. **115**: 495–501.
- Menge, J.A., Johnson, E.L.V. and Platt, R.G. (1978). Mycorrhizal dependency of several citrus cultivars under three nutrient regimes. *New Phytol.* **81**: 553–560.
- Muthukumar, T. and Udaiyan, K. (2002). Seasonality of vesicular-arbuscular mycorrhizae in sedges in a semi-arid tropical grassland. *Acta Oecol.* **23**: 337–347.
- Muthukumar, T., Sha, L., Yang, X., Cao, M., Tang, J. and Zheng, Z. (2003). Mycorrhiza of plants in different vegetation types in tropical ecosystems of Xishuangbanna, southwest China. *Mycorrhiza* **13**: 289–297.
- Nair, P.K.R. (1993). **An Introduction to Agroforestry**. Kluwer Academic Publishers, Dordrecht.
- NMA (National Meteorological Agency of Ethiopia) (2010). Annual climate bulletin for the year 2002-2010.
- Omar, M.B., Bolland, L. and Heather, W.A. (1979). PVA (polyvinyl alcohol). A permanent mounting medium for fungi. *Bull. Brit. Mycol. Soc*. **13**: 31–32.
- Sawers, R.J.H., Yang, S-Y., Gutjahr, C. and Paszkowski, U. (2008). The molecular components of nutrient exchange in arbuscular mycorrhizal interactions. In: **Mycorrhizae: Sustainable Agriculture and Forestry**, pp. 37–59 (Siddiqui, Z. A., Akhtar, M.S. and Kazuyoshi Futai, K., eds). Springer Science + Business Media B.V.
- Schenck, N.C. and Perez, Y. (1990). **Manual for the Identification of VA Mycorrhizal Fungi**. Third ed. Synergistic publications, Gainesville, Fla.
- Schüßler, A. and Walker, C. (2010). The Glomeromycota. A species list with new families and new genera. Published in December 2010 in libraries at The Royal Botanic Garden Edinburgh, The Royal Botanic Garden Kew, Botanische Staatssammlung Munich, and Oregon State University. Electronic version freely available at

www.amf-phylogeny.com.

- Smith, S.E., and Read, D.J. (2008). **Mycorrhizal Symbiosis**. Third ed. Academic Press, London.
- Stutz, J.C. and Morton, J.B. (1996). Successive pot cultures reveal high species richness of arbuscular endomycorrhizal fungi in arid ecosystems. *Can. J. Bot*. **74**: 1883–1889.
- Stutz, J.C., Copeman, R., Martin, C.A. and Morton, J.B. (2000). Patterns of species composition and distribution of arbuscular mycorrhizal fungi in arid regions of southwestern North America and Namibia, Africa. *Can. J. Bot*. **78**: 237–245.
- Tadesse Chanie and Fassil Assefa (2013). Arbuscular mycorrhizal fungi associated with shade trees and *Coffea arabica* L. in a coffee-based agroforestry system in Bonga, Southwestern Ethiopia. *Afr. Foc.* **26**: 11–131.
- Trindade, A.V., Siqueira, J.O. and Stürmer, S.L. (2006). Arbuscular mycorrrhizal fungi in papaya plantations of Espirito Santo and Bahia, Brazil. *Braz. J. Microbiol*. **37**: 283 –289.
- Wehner, J., Antunes, P.M., Powell, J.R., Mazukatow, J. and Rillig, M.C. (2010). Plant pathogen protection by arbuscular mycorrhizas: A role for fungal diversity? *Pedobiologia* **53**: 197–201.
- Yonas Yohannes and Fassil Assefa (2007). Phenotypic characteristics of root nodule bacteria and arbuscular mycorrhizal fungi infecting *Acacia polyacantha* growing in Ghibe wooded grasslands. *Ethiop. J. Nat. Sci.* **9**(1): 123–139.
- Zhao, D.D. and Zhao, Z.W. (2007). Biodiversity of arbuscular mycorrhizal fungi in the hot-dry valley of the Jinsha River, Southwest China. *Appl. Soil Ecol*. **37**: 118–128.