academicJournals

Vol. 8(38), pp. 3489-3500, 17 September, 2014
DOI: 10.5897/AJMR2013.6446
Article Number: 24401FD48000
ISSN 1996-0808

http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Effect of variation in land use, age of host tree, season and geographic location on the diversity of endophytic fungi in the needles of *Afrocarpus falcatus*

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Received 23 October, 2013; Accepted 8 September, 2014

The aim of the current study was to investigate the effect of variation in land use, age of host tree, season and geographic location on the diversity of culturable endophytic fungi of needles of *Afrocarpus falcatus*. Endophytic fungi represent a genetically diverse group of microorganisms associated with healthy tissues of terrestrial plants. They are believed to be mostly beneficial to their host plants and produce novel antimicrobial compounds; or may be latent pathogens that become active at specific stage of development or under a set of environmental conditions. Wondo Genet and Menagesha Suba were the two geographic locations with differing altitudinal and climatic features selected for sample collection. Needle samples were collected from old and young trees growing in natural forests and open lands during dry and wet seasons. Identification of the isolates to the genus level was performed on the basis of culture characteristics and spore morphology. A total of 687 endophytic fungal isolates were obtained and categorized into 64 morphotaxa. Fifty two (81.25%) of the morphotaxa were identified to 12 genera while 12 morphotaxa were left as unidentified. Thirty two (50%) of the morphotaxa were isolated only from the Wondo Genet site while only 3 (4.7%) were isolated from the Menagesha Suba site. The four most diverse genera were *Cercosporella, Xylaria, Botryosphaeria* and *Pestalotiopsis*. *Phoma* was the most abundantly represented genus followed by *Xylaria* and *Pestalotiopsis* while *Trichotecium* and *Mycosphaerella* were the rarest genera. The Wondo Genet site during the dry season was the most diverse (H'= 3.36649) and many of the morphotaxa were unique to the site; which might be ascribed to the differences in altitudinal, climatic and perhaps additional factors that were not considered in the current study.

Key words: Fungal diversity, morphotaxa, *Afrocarpus falcatus.*

INTRODUCTION

The term endophyte belongs to those groups of microorganisms that inhabit the living internal tissue of green plants without causing immediate or clear effect on the plant (Hirsch and Brauna, 1992) and may cause a clear negative effect when the plant faces some kind of environmental stress. Endophytic fungi are those groups

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of fungi that live in the internal tissue of living plants and are important component of fungal diversity and affect plant community diversity and structure. According to different authors, the number of endophytic fungi inhabiting plants tissues is estimated to be about 1 million (Dreyfuss and Chapela, 1994).

Endophytic fungi serve as a biocontrol of plant pathogen by actively inhibiting pathogens from invading the host plant. This is consistent with the observation of Herre et al. (2005), that in the vast majority of cases, endophytes appear not to harm their host plant, and not to reproduce. This helps the host plant to cope with (withstand) the damage caused by pathogens. The presence of endophytes in plants enhances host defense against pathogenic infection and reduce the damage due to herbivores (Herre et al., 2005) thus improving the existence of the host plant.

According to Arnold and Herre (2003), the presence of endophytes in the host substantially reduces leaf loss and damage due to an oomycete pathogen. In addition to increasing host defense against pathogens and herbivores, it has been also reported that some species of endophytic fungi increases drought and heavy metal tolerance. Furthermore, an endophytic fungus *Theobroma cacao* was reported to contribute to the host stress tolerance from ecosystem by affecting levels of photosynthesis and hydraulic properties (Herre et al., 2005).

Almost all green plants harbor neutral endophytes but because they are symptomless and are difficult to detect and can only be successfully surveyed by plating out carefully prepared surface-sterilized tissues. For example, a study by Fisher and Petrini (1990) on the endophytes isolated from *Alnus* xylem and barks tissues in roots and stems yielded 85 different fungal taxa (Fisher and Petrini, 1990).

Many of the endophytic fungi remain quiescent (inactive) within their hosts until the host is stressed or begins to undergo senescence. An extreme example of this type of endophyte is the fungi occurring in conifer needles (Dix and Webster, 1995).

Given the many ecological benefits of endophytic fungi to their host plants and to the socio-economic development of nations, there were no enough studies on endophytic fungi in Ethiopia in general and on forest trees in particular. Not only their diversity but also their distribution within and among host plants under different ecological situations needs to be explored.

The current study attempted to examine the diversity of endophytic fungal assemblages of *A. falcatus* trees growing in different geographical locations having different altitudinal ranges and land use systems. If there occur any variation between the aforementioned parameters it was meant to show if they have some impacts on the diversity of endophytic fungi in the needles.

The objective of this study was to assess the diversity of endophytic fungi associated with needles of *A. falcatus* in relation with season, land use, age of host tree and geographic variation. Simultaneously, this research work has an aim to isolate and morphologically categorize culturable endophytic fungi inhabiting needles of *A. falcatus* collected from natural forests and open lands at Wondo Genet and Menagesha Suba. Finally, the research aims to assess the impact of age of host, seasonal, altitudinal and land use variations on the diversity of endophytic fungi inhabiting the needles of *A. falcatus*.

METHODOLOGY

Description of the study area

The study was conducted at two geographic locations, namely Wondo Genet and Menagesha Suba that are over 300 km apart. Moreover, they are believed to have, among others, different altitudinal ranges and climatic factors.

Wondo Genet

Geographical location: Wondo Genet is situated 263 km South of Addis Ababa and 13 km southeast from the nearest town, Shashemene, West Arsi Zone in Oromia Regional State and about 38 km from Hawassa town. It is located between 38°37'-38° 42'E longitude and 7°02'-7°07'N latitude and an altitude of between 1600 and 2500 masl. The study was conducted in the plantations and natural forests found in the premises of Wondo Genet College of Forestry and Natural Resources (http://www.wgcf-nr.org/resource/land.html).

Climatic condition of Wondo Genet is traditionally categorized under Weyna dega. Wondo Genet has a bimodal rainfall pattern. The 'short' rains generally arrive in mid- to late February, while the 'long' rains begin late June and continue to the end of September. Occasional, sporadic rain occurs during the dry seasons, but it has little impact on the growth patterns of the plants. Annual rainfall ranges from approximately 700 to 1400 mm. The mean annual temperature at the College campus is 19°C. The area has a high population density of 588 people per km^2 and has an estimated total population of 5,792 consisting of 2,857 men and 2,935 women (CSA, 2005).

The forest of Wondo Genet constitutes both the natural and plantation forests. The natural forest constitutes an important pocket that is disturbed but still in good condition with its plant and animal species protected from the impacts of human beings in relation to many of the remaining natural forests in the country. Some of the major tree species in the natural forest include *Albizia gummifera*, *Celtis africana*, *Cordia africana*, *Croton macrostachyus*, *Prunus africana* and *Afrocarpus falcatus* (*Podocarpus falcatus* syn.), *Aningeria adolfi-friederici*, scattered *Acacia* trees and rank tall grasses are found in this area (http://www.wgcf-nr.org/resource/land.html).

Menagesha Suba Forest

Menagesha Suba forest is located 50 km south-west of Addis Ababa at 38°31'-38035'E longitude and 8°56'-$9^0$4'N latitude (Teketay, 1996) with an altitude ranging between 2200 to 3000 masl. The area consists of an isolated mountain.

The natural forest communities of Menagesha Suba consisted of Hypericum belts, Hagenia-juniperus forest, Juniperus forest, Juniperus-Afrocarpus forest and Afrocarpus forest in descending order of elevation (from 3500 m down to 2000 m) (Feyera and Demel, 2001).

Climatic condition of Menagesha Suba is traditionally categorized as 'Dega' to 'Weyna Dega'. The annual temperature of the area is between 15-17°C and has an annual rainfall of 1100 mm (Fetene, 2006).

Sample collection

Sample collection was carried out during the period from June to August 2010 to cover the wet season and from November to January, 2010 to represent the dry season. In both sites (Wondo Genet and Menagesha Suba), two land use types (natural forest and open lands such as parks and farm lands) were selected. In both land use types, three young (having dbh 13.5 - 16.5 cm) and three old (dbh 68-90cm) trees of *A. falcatus* were randomly selected for needle sampling.

From each sample tree, 4 healthy looking (asymptomatic) needles were randomly picked from bottom, middle and top parts of the crown of a tree. Thus, from each site, a total of 12 needles per age group and 24 per land use type and season and 192 needles in total were collected for isolation.

The needle samples were collected in paper bags, labeled and transported to the laboratory immediately upon collection for isolation (Barik et al., 2010). Samples from Menagesha Suba were transported to the laboratory within 24 h after collection with intermittent wetting with water to protect the needles from drying.

Nutrient media preparation

A 2% malt extract agar (MEA) was used to isolate fungi. For plate culture, the sterilized MEA was poured onto Petri dishes for both primary and pure culture.

After the needle samples were cut into 10 mm fragments, the fragments were briefly sprayed with 70% ethanol before being transferred to 3% sodium hypochlorite (NaOCl) for surface sterilization (Guo et al., 2001). The ethanol acts as a surfactant and the NaOCl is the actual sterilizing agent (Bills, 1996).

They were then washed five times consecutively. The surface sterilized needles were then dried by blotting on sterile filter paper under aseptic condition. Four surface sterilized needle fragments per plate were randomly picked and plated on MEA plates and incubated at 25°C for 4-10 days to allow growth of fungal colonies on the medium. Pure cultures were obtained by sub-culturing them on MEA medium and maintained on slants tubes containing the same medium.

Identification of fungi

Identification was based upon cultural and microscopic (conidial) characteristics. The cultural characteristics included the color of the upper and reverse sides of the cultures, mycelial color formation such as dark, brown, grey, yellow or other colors, colony diameter, shape of colony margin, mycelial growth patterns such as fluffy aerial hyphae, appresssed or submerged hyphae, formation of aerial hair-like tufts of hyphae were used to characterize the fungal endophytes.

Based on culture characteristics and spore morphology, the isolated fungi were categorized into morphotaxa identified to the genus level, while those which could be separated into distinct groups based on culture characteristics but could not be identified to any of the known genera were recognized as unidentified taxa. Lactophenol cotton blue staining solution was used for staining of non-pigmented fungal spore for microscopic examination purpose. Conidial morphological characteristics including shape and color of the spores, separation, presence or absence of specialized appendages on the spores were used to characterize the fungal structure.

In addition, different standard identification manuals were used to provisionally identify the fungal isolates to the genus level. Whenever more than one morphological group occurs within a genus, they were designated as sp.1, 2, 3, etc.

Data analysis and presentations

Data obtained from the research work was presented using tables as percentages. Colonization rate (CR, expressed as a percentage) was calculated as the total number of needle segments infected by fungi divided by the total number of needle segments incubated and it was used to compare the degree of infection by endophytic fungi of needle tissues collected from host trees under different land use, age group and geographic location (Lv et al., 2010).

$$CR = \frac{\text{Total number of needle segment incubated}}{\text{The number of needle segments infected by fungi}} \times 100$$

The isolation rate (IR, is a quotient calculated by dividing the number of isolates obtained from needle segments by the total number of needle segments incubated. This allows for the measurement of fungal species richness in a needle (Lv et al., 2010). IR was calculated according to the equation below:

$$IR = \frac{\text{Number of isolates obtained from plant segment}}{\text{Total number of plant segmented incubated}}$$

The relative frequency (RF, expressed as a percentage) was calculated as the total number of isolate from a single taxa divided by the total number of isolates from taxa obtained from all tissue incubated (Lv et al., 2010). It was used to determine the most frequently isolated taxa among the rest of taxa.

$$RF = \frac{\text{Number of isolates of a taxon}}{\text{Total number of isolates of all taxa}}$$

Additionally, both the Shannon diversity index and Sorensen Similarity index was calculated for the two sites. Shannon diversity index (*H'*) was used to characterize species diversity in a community.

Shannon index of diversity was calculated for all factors which were assumed to have effect on the diversity of endophytic fungi. To check the significance level of the variations ANOVA was employed using SPSS version 20 used at $\alpha = 0.05$.

RESULTS AND DISCUSSION

A total of 687 endophytic fungal isolates were recovered from 192 needles (960 needle fragments) collected from all sampling points (land uses, age of host trees, geographic location and season). The distribution of endophytic fungal isolates between the two sampling sites showed that those trees from Wondo Genet were found to harbor the highest number (53.6%); whereas

Table 1. Total number of endophytic fungal isolates recovered from needles of *A. falcatus* trees from different geographic locations, land uses and seasons.

Land use	Wondo Genet		Menagesha Suba		Total
	Wet season	Dry season	Wet season	Dry season	
Open Land	90	79	100	52	321
Natural forest	86	113	94	73	366
Total	176	192	194	125	687
	368		319		

Table 2. Total number of endophytic fungal isolates recovered from needles of *A. falcatus* trees from different geographic locations, land uses and different host tree age group.

Land use	Wondo Genet		Menagesha Suba		Total
	Young	Old	Young	Old	
Open Land	90	79	82	70	321
Natural forest	103	96	80	87	366
Total	193	175	162	157	**687**

those from Menagesha Suba samples harbor the lower number of isolates (46.4%) of isolates (Table 1). However, the data is not statistically significant at P>0.05. Within each geographic location, varying number of endophytic fungal isolates were recovered from the two land uses (open land and natural forest) during wet and dry seasons (Table 1), this was because that higher diversity of plant life in natural forest and fungal abundance decreases as altitude increases (Brosi et al., 2011; Kodsueb et al., 2008).

The highest number of endophytic fungal isolates (113) was recovered from needles collected from natural forest at Wondo Genet during the dry season while the lowest number of isolates (52) was recovered from needles collected from open land trees at Menagesha Suba during the dry season (Table 1). The previous study by Talley et al. (2002), Gamboa and Bayman (2001) and Rojas and Stephenson (2008) strengthen our finding that the abundance of endophytic fungi is higher in undisturbed forest community, it is due to the high inoculums by endophytic fungal spore arising from the diverse array of plant life.

The difference between open land and natural forest trees in the number of endophytic fungal isolates recovered was higher during the dry season both at Wondo Genet and Menagesha Suba, although the difference was not statistically significant at P>0.05. This might be due to the high water stress in wet season that leads to the lowest number of endophytic fungi in both sampling sites.

It also appears from the results of the current study that young trees generally harbor more endophytic fungal isolates than old ones but the data was not statistically significant at P>0.05 in Wondo Genet and in Menagesha Suba (Table 2). The current observation concurs with those of Helander et al. (2006) stating that younger plants harbor higher number of endophytic fungi than older ones. This might be due to the soft leaf chemistry of younger plants. Furthermore, it appears that a combination of seasonal variation, age of host tree and land use type influences the abundance of endophytic fungal communities associated with the needles of *A. falcatus* (Table 2).

Isolation attempts from open lands trees generated more number of fungal isolates during the wet season than during the dry season at both Wondo Genet (90) and Menagesha Suba (100), whereas higher number of isolates were recovered from natural forest trees during the dry season at Wondo Genet (113) and from natural forest during the wet season at Menagesha Suba (94) (Table 1).

The decrease in the number of isolates from natural forests during the wet season and the higher number of isolates during wet season from open land trees might be explained by the excess moisture in the natural forests that could be a stress for the endophytic fungi associated with the needles of *A. falcatus* (Talley et al., 2002). This could also explain the higher number of isolates from natural forest trees but lower number of isolates from the open lands during the dry season in both geographic locations (Table 1).

The general comparison of land uses against age of host trees indicated that more endophytic fungal isolates were recovered from natural forest (366) than open land (321) considering both the geographic locations together. This may be related to the higher plant community diver-

Table 3. Distribution of the morphotaxa in all the sampling points in both seasons and sites.

Season	Sampling site and land use	Morphotaxa	RF (%)
Wet	Wondo Genet Open land	33	26%
	Wondo Genet Natural forest	47	36.5
	Menagesha Suba open land	10	8
	Menagesha Suba Natural forest	13	10
Dry	Wondo Genet Open land	37	29
	Wondo Genet natural forest	54	42
	Menagesha Suba Open land	22	17.5
	Menagesha Suba natural forest	28	21.5

RF: Relative frequency.

Table 4. Distribution of morphotaxa of endophytic fungal isolates of Wondo Genet and Menagesha Suba sites in both seasons.

Season	Sampling sites	Morphotaxa recovered	RF (%)
Wet	Wondo Genet	45	70
	Menagesha Suba	9	14
Dry	Wondo Genet	47	73
	Menagesha Suba	30	47

RF: Relative frequency.

sity of natural forest than that of open land habitat (Tsui et al., 1998; Aung et al., 2008; Gamboa and Bayman, 2001; Rodrigues, 1994), which may serve as a potential source of inoculums. In addition**,** natural forests at both study sites are relatively well protected and richer in plant communities and thus more diverse in endophytic fungal communities than the isolated trees in the open lands. The vegetation that surrounds it is believed to be an important source of inoculums for endophytes (Gamboa and Bayman, 2001; Rodrigues, 1994).

Likewise, isolation attempts from young trees generally recovered more endophytic fungal isolates than from old trees (Table 2). Moreover, needles of young trees from natural forest at Wondo Genet yielded more endophytic fungal isolates than those from open land trees. However, young trees from natural forest at Menagesha Suba yielded slightly lower number of isolates than those from open land (Table 2); but the difference was not statistically significant at $P>0.05$. Additionally, young trees, except from natural forest at Menagesha Suba, yielded more number of isolates than old trees. The result of the current study was in agreement with the finding of Helander et al. (2006).

The endophytic fungal isolates were grouped into 64 morphotaxa. From the geographic locations, isolations from Wondo Genet during the dry season recovered more morphotaxa (73%), followed by wet season Wondo Genet (70%) of the morphotaxa (Table 4). The lower number of morphotaxa was recovered in both the dry and wet season in Menagesha Suba as compared to Wondo Genet.

The results of the current study were in agreement with the findings of Osono and Hirose (2009) and Hashizume et al. (2010) which stated that diversity of endophytic fungi decreases with increase with altitude and vice versa.

The largest number of morphotaxa **(**48.43%) was isolated from needles from young trees in the natural forest at Wondo Genet during the dry season followed by trees from the same site but during the wet season **(**42.19%) (Table 3). Similarly, at Menagesha Suba, the highest number of morphotaxa was recovered from young trees from natural forest during both the dry and wet seasons. Generally, young trees in the natural forest yielded the highest number of morphotaxa. Helander et al. (2006) also observed similar pattern of association of endophytic fungi with young trees than in the old trees and in the undisturbed forest than in disturbed forest. This pattern of distribution was consistent with the abundance of the endophytic fungal isolates (Tables 1 and 2).

Out of the 64 morphotaxa, 32 (50%) were unique to

Table 5. Genera encompassing the morphotaxa of endophytic fungi isolated from needles of *A. falcatus* from Wondo Genet and Menagesha Suba sites.

Genera	No. of morphotaxa	No of isolates	Origin				RF (%)
			WG		MS		
			Wet	Dry	Wet	Dry	
Botryosphaeria spp.	7	50	√	√	√	√	10.93
Pestalotiopsis spp.	5	65	√	√	√	√	7.81
Xylaria spp.	9	70	√	√	√	√	14.06
Phoma spp.	4	219	√	√	√	√	6.25
Cercosporella spp.	10	53	√	√	√	√	15.62
Fusarium spp.	3	13	√	X	√	√	4.65
Microsphaeropsis spp.	2	5	√	√	X	√	3.12
Chaetomium spp.	3	7	√	√	X	√	4.68
Aspergillus spp.	5	53	√	√	√	√	7.81
Trichotecium sp.	1	1	√	X	X	X	1.56
Penicillium spp.	2	54	√	√	X	√	3.12
Mycosphaerella sp.	1	2	X	√	X	X	1.56
Unidentified groups	12	95	√	√	X	√	18.75
Total	**64**	**687**					

√-Present, X- absent, WG-Wondo Genet, MS-Menagesha Suba, RF- Relative frequency.

Wondo Genet while only 3 (4.7%) were unique to Menagesha Suba. Moreover, 61 of the 64 morphotaxa were recorded at Wondo Genet while only 50% (32 of the 64) morphotaxa were recorded from Menagesha Suba. This clearly indicates that the two geographic locations are significantly different not only in terms of species richness and abundance but also due to the proportion of unique taxa they harbor. This observation may be due to several factors that may have been involved, most notably the geographic and climatic variations between the two sites. Furthermore, genetic variations of the host trees and the floristic composition of the sites may have contributed to the observed differences. However, such speculations need to be verified with further studies.

Comparing the seasons, dry season was better for recovering higher number of endophytic fungal morphotaxa than the wet season at both study sites, that is, 47 of the 64 in dry season at Wondo Genet and Menagesha Suba 30 of the 64 (Table 4). The highest proportion of morphotaxa were recovered during the dry season at Wondo Genet from young trees in the natural forest (48%) followed by the same site but during the wet season (42%) (Table 3).

Likewise, the lowest proportion of morphotaxa were recovered during the wet season from trees in open land (8%) (Table 3). This may indicate that differences in endophytic fungal diversity may be influenced by altitudinal and seasonal variations. The findings of the current study were in agreement with those of Brosi et al. (2011), Talley et al. (2002) and Kodsueb et al. (2008). The decrease in temperature and high water stress leads to decrease in endophytic fungal diversity in wet season (Krishnamurthy et al., 2009). Rojas and Stephenson (2008) also concluded that endophytic fungal distribution decreases as altitude increases and vice versa; and natural forests harbor higher fungal diversity than the open land (Tsui et al., 1998).

Identification of the morphotaxa

Among the identified morphotaxa, the four most diverse (species rich) genera were *Cercosporella, Xylaria*, *Botryosphaeria* and *Pestalotiopsis*, represented by 10, 9, 7 and 5 morphotaxa, respectively. Similarly, the three most abundant genera were *Phoma*, *Xylaria* and *Pestalotiopsis*, represented by 219, 70 and 65 isolates, respectively while *Trichotecium* and *Mycosphaerella* were the least diverse and the rarest genera among the endophytic fungal communities encountered during the current study (Table 5). Furthermore, *Mycosphaerella* and *Trichotecium* were not isolated from Menagesha Suba site during both seasons. Moreover, the unidentified genera were not isolated from Menagesha Suba site during the wet season. The rest of the morphotaxa were encountered at both the study sites. However, 12 morphotaxa could not be identified to the genus level as they did not sporulate under the laboratory conditions of the current study. They accounted for about 18% (95 isolates) of the collection (Table 5).

In general, comparison, higher degree of colonization rate (CR) by fungal endophyte was found at Wondo

Table 6. Number of needle segment that yielded one or more fungal isolates, total no. of needle segment that incubated the colonization rate and isolation rate in both seasons at both sampling sites.

Sample points	No. of needle segments that yielded one or more fungal isolates	CR (%)	IR
WWOLY	52	86.67	0.8
WWOLO	43	71.67	0.7
WWNFY	49	81.67	0.6834
WWNFO	45	75	0.75
DWOLY	49	81.67	0.7
DWOLO	40	66.67	0.6167
DWNFY	53	88.34	1.034
DWNFO	56	93.34	0.85
WMOLY	48	80	0.967
WMOLO	40	66.67	0.7
WMNFY	60	100	0.7834
WMNFO	55	91.67	0.7834
DMOLY	21	35	0.4
DMOLO	29	48.34	0.467
DMNFY	20	33.34	0.55
DMNFO	30	50	0.667

DMNFO, Dry season Menagesha Suba natural forest older; DMNFY, Dry season Menagesha Suba natural forest younger; DMOLO, Dry season Menagesha Suba open land older; DMOLY, Dry season Menagesha Suba open land younger; DWNFY, Dry season Wondo Genet natural forest younger; DWNFO, Dry season Wondo Genet natural forest older; DWOLY, Dry season Wondo Genet open land younger; DWOLO, Dry season Wondo Genet open land older; WMNFO, Wet season Menagesha Suba natural forest older; WMNFY, Wet season Menagesha Suba natural forest younger; WMOLO, Wet season Menagesha Suba open land older; WMOLY, Wet season Menagesha Suba open land younger; WWNFO, Wet season Wondo Genet natural forest older; WWNFY, Wet season Wondo Genet natural forest younger; WWOLO, Wet season Wondo Genet open land older; WWOLY, Wet season Wondo Genet open land younger.

Genet than Menagesha Suba, natural forest than open land and on young trees than old trees (Table 6). Similarly, in general, higher isolation rate (IR) by fungal endophytes was recovered at Wondo Genet than Menagesha Suba and from natural forest than open land (Table 6). This result is also in agreement with abundance of endophytic fungi in Tables 1 and 2. The previous studies by Granath et al. (2007) and Raviraja et al. (1998) have indicated that species richness is greater during the dry season than the wet season and also claimed that species richness decreases with increase in altitude.

The highest diversity index (*H'*=3.01856) of endophytic fungi was calculated for DWNFY followed by DWOLY (*H'*=3.0055), while the lowest diversity index was obtained for WMOLO (H'=1.004) (Figure 1). The results of the current study are in agreement with findings of Rojas and Stephenson (2008), Hashizume et al. (2008) and Arnold and Lutzoni (2007) strengthening the claim that diversity of endophytic fungal species decreases with the increasing altitude.

This result is also in accordance with the previous observations on the species richness and abundance in the same study. The highest diversity of endophytic fungi was recovered during the dry season than wet season both at Wondo Genet and Menagesha Suba. It is also in accordance with the findings of Brosi et al. (2011) where it indicated that diversity of endophytic fungi was higher during the dry season. The lower *H'* index for Menagesha Suba was influenced by the altitude (Hashizume et al., 2008; Higgins et al., 2007).

The dominant morphogenera at Wondo Genet during the wet season from old trees was *Botryosphaeria* spp. (35.7%); but the dominant morphogenera from samples in open land at old trees at Wondo Genet during the wet season was *Xylaria* spp. (22.58%). However, the dominant morphogenera from open land older trees at Menagesha Suba during the wet season was *Phoma* spp. (85.7%).

The dominantly isolated morphotaxon among the rest of taxa at Wondo Genet from open land young trees during the wet season was *Penicillium* sp. 2 (37.5%)

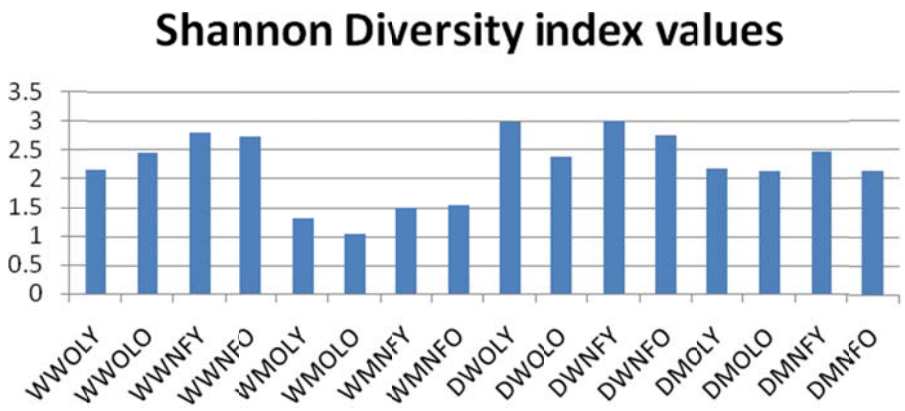

Figure 1. Shannon diversity index for all the sampling points in both sampling sites. Full explanation of the abbreviation is shown in the footnote of Table 6.

followed by *Phoma* sp. 4 (14.58%). However, in the same season and site but from older trees the dominantly isolated morphotaxon during the wet season was *Botryosphaeria* sp. 7 (28.57%) followed by *Xylaria* sp. 9 (11.9%). Indicating that in the same season, site and land use but different ages, there is difference in the type of dominant species.

Similarly, in wet season at Wondo Genet, the dominantly isolated morphotaxa from natural forest younger *A. falcatus* was *Aspergillus* sp. 4 (19.51%); however in the same season and site, the dominantly isolated morphotaxon from natural forest older *A. falcatus* was *Cercosporella* sp. 5 (20%) followed by *Phoma* sp. 4 (11.11%).

The dominantly isolated morphotaxon in dry season from open land at Wondo Genet from both younger and older *A .falcatus* was different, that is, from younger *A. falcatus* was *Pestalotiopsis* sp. 4 (11.9%) and that of older *A. falcatus* was *Aspergillus* sp. 2 (27.03%), indicating that there occur difference dominancy of taxa between age. However, the dominantly isolated morphotaxon in dry season from natural forest at Wondo Genet was *Pestalotiopsis* sp. 4 (19.35% and 15.68%) from younger and older *A. falcatus*, respectively.

The dominant morphotaxon at wet season in Menagesha Suba from open land younger and older *A. falcatus* was *Phoma* sp. 1 (55.17 and 61.9% respectively). However, in wet season, the dominant morphotaxon from natural forest younger *A. falcatus* was *Phoma* sp. 1 (42.55%) but the dominant taxa in the same season at Menagesha Suba from natural forest older *A. falcatus* was *Phoma* sp. 3 (42.55%).

In dry season at Menagesha Suba site in both open land and natural forest, there was difference in the type of dominant morphotaxon. Generally, in the result obtained from Table 6, there is difference in dominant morphotaxon between ages of host, season of sampling and altitude (Suryanarayanan et al., 2002).

Species diversity

The Shannon diversity index for seasonal variation on endophytic fungi shows that higher diversity of endophytic fungi was recovered at Wondo Genet site during the dry season (*H'* = 3.36649) than during the wet season (*H'*= 3.35275) but it was statistically not significant at P>0.05. This is because the high water stress and low temperature in wet season decrease the diversity of endophytic fungi (Gonthier et al., 2006; Talley et al., 2002). This result was also in accordance with the previous study by Hashizume et al. (2008) that is, the diversity endophytic fungi increases with increasing temperature during the dry season.

Similarly, at Menagesha Suba site higher endophytic fungi diversity was recovered during the dry season (*H'*= 2.65877) than wet season (*H'*= 1.47950), this is because the diversity of endophytic fungi is higher in dry season (higher in temperature) (Brosi et al., (2011; Vacher et al., 2008 and Compant et al., 2010) than wet season due to high water stress present (Gonthier et al., 2006), which is also statistically not significant at P>0.05.

In accordance with the work of Osono and Hirose (2009) and Hashizume et al. (2008) higher diversity of endophytic fungi diversity was found higher at Wondo Genet site than Menagesha Suba forest in the same season.

The Shannon diversity index for geographic variation between sampling sites indicates that the diversity of endophytic fungi was numerically higher at Wondo Genet site (*H'*= 3.35275) than from trees at Menagesha Suba site (*H'*= 1.47950) during the wet season, but the data was statistically not significant at P>0.05, indicating that the diversity of endophytic fungi decreases as altitude increases (Rojas and Stephenson, 2008; Talley et al., 2002).

Similarly, the diversity of endophytic fungi at Wondo Genet site (*H'*=3.36649) was higher than samples at

Table 7. Shannon diversity index for endophytic fungi isolated from *A. falcatus* tree of different age group.

Sampling site and season	*H'*
Wondo Genet at wet season (young trees)	2.85575
Wondo Genet at wet season (old trees)	3.16488
Wondo Genet at dry season(young trees)	3.29748
Wondo Genet at dry season (old trees)	3.01591
Menagesha Suba at wet season (young trees)	1.44382
Menagesha Suba at wet season (old trees)	1.41303
Menagesha Suba at dry season (young trees)	2.55959
Menagesha Suba at dry season (old tree)	2.29078

Table 8. Shannon diversity index for different land uses of each sampling site and season.

Sampling site and season	*H'*
Wondo Genet at wet season (from open land)	2.82897
Wondo Genet at wet season (from natural forest)	3.20512
Wondo Genet at dry season (from open land)	3.11148
Wondo Genet at dry season (from natural forest)	3.09124
Menagesha Suba at wet season (from open land)	1.23645
Menagesha Suba at wet season (from natural forest)	1.61325
Menagesha Suba at dry season (from open land)	2.50492
Menagesha Suba at dry season (from natural forest)	2.50277

Menagesha Suba site (*H'*=2.65877) during the dry season, but it is statistically not significant at P>0.05. The previous study by Rojas and Stephenson (2008) and Arnold and Lutzoni (2007) also indicates that as altitude increases the diversity of endophytic fungi decreases and vice versa.

At Wondo Genet site during both dry and wet seasons, the diversity of endophytic fungi was nume-rically higher than what was observed during both seasons at Menagesha Suba site, indicating that species richness and diversity increases as a function of decreasing latitude (Arnold and Lutzoni, 2007; Rojas and Stephenson, 2008; Hawksworth, 1991) but the data was not statistically significant at P>0.05.

In accordance with the finding of Wang and Guo (2007) the diversity of endophytic fungi was numerically higher than older trees at Wondo Genet during the wet season (*H'*=3.16488) than younger trees (*H'*= 2.85575) (Table 7), but it was statistically not significant at P>0.05.

However, the Shannon diversity index for endophytic fungi isolated from young trees at Wondo Genet during the dry season was higher on young tree than that of samples from old trees at the same site (Table 8). This result was in agreement with the finding of Helander et al. (2006) indicating that young plants harbor higher number of fungi than old ones.

The diversity of endophytic fungi was higher for young tress than old trees at Menagesha Suba during both the dry and wet seasons, but the data is statistically not significant at P>0.05. The results of the current study are also in agreement with those of Helander et al. (2006).

In accordance with the finding of Tsui et al. (1998), the diversity of endophytic fungi in both sampling sites during the wet season was higher than samples collected from natural forest trees (Table 8). This is because natural forest have higher plant diversity (Helander et al., (2006) that is, surrounding vegetation is an important source of inoculums for endophytes (Gamboa and Bayman, 2001; Rodrigues, 1994) but the data is not statistically significant.

However, the diversity of endophytic fungi in dry season both at Wondo Genet and Menagesha Suba site was found to be higher than samples collected from open land *A. falcatus* and that of natural forest (Table 8). This finding was in accordance with the finding of Aung et al. (2008), indicating that the diversity of endophytic fungi is higher in disturbed forest. This might be due to the high disturbance; dust formation and wind movement in dry season serve trees in open land to get more inoculants than natural forest.

Table 9. Sorensen similarity index between seasons in the same land use.

Sampling site	Season	Total number of isolates	No. of commonly shared isolates	*QS*
WG	Wet	176	25	0.1358696
	Dry	192		
MS	Wet	194	3	0.0188088
	Dry	125		

QS: Sorensen similarity index, WG- Wondo Genet, MS- Managesha Suba.

Table 10. Number of isolates of each land use systems, number of commonly shared isolates and Sorensen similarity index.

Site and season	Land use	Total no. of isolates	No. of commonly shared isolates	*QS*
WG at Wet season	Open land	90	15	0.1704545
	Natural Forest	86		
WG at Dry season	Open land	79	20	0.2083333
	Natural Forest	113		
MS at Wet season	Open land	100	6	0.0618557
	Natural Forest	94		
MS at Dry season	Open land	52	10	0.16
	Natural Forest	47		

WG-Wondo Genet, MS-Managesha Suba.

Species similarity

Species similarity between sampling units was calculated using Sorensen similarity index. The highest similarity between sampling seasons was found at Wondo Genet (*QS*=0.1358696) than the similarity index value observed at Menagesha Suba site (*QS*= 0.0188088) (Table 9), which is in contrast with the previous finding by Wang and Guo (2007) stating that composition of endophyte assemblages is not greatly influenced by geographical or climatic factors. In addition, the similarity between seasons at Wondo Genet site was higher than Menagesha Suba site. The result, also, suggests that the similarity between sampling seasons decreases as altitude increases (Table 9).

The highest similarity between land use systems was found at Wondo Genet site during the dry season (*QS*= 0.2083333) than during the wet season (*QS*=0.1704545) (Table 10). Similarly, the similarity between land use systems at Menagesha Suba site was numerically higher during the dry season (*QS*=0.16) than during the wet season (*QS*=0.0618557) (Table 10). Indicating that the similarity between land use systems during both seasons at Wondo Genet site was higher than what was observed during both seasons at Menagesha Suba site (Table 10). The lowest number of commonly shared isolates (6) between seasons was recovered at Menagesha Suba and indicating that higher moisture difference between the two sampling seasons at Menagesha Suba. This result is in agreement with the finding of Stanwood (2009).

The Sorensen similarity index for age of host tree indicates that at Wondo Genet site the similarity between age groups was numerically higher during the dry season than (*QS*= 0.1979167) than during the wet season (0.1704545) (Table 11), this is also in contrast with the previous finding by Wang and Guo (2007). Similarly, the Sorensen similarity index between age groups at Menagesha Suba site was found to be numerically higher in dry season (*QS*=0.144) than wet season (0.039278). These results suggest that as altitude increases the similarity between age groups decreases. As altitude increases, the similarity between age groups in the same season was found to be higher in Wondo Genet than Menagesha Suba site.

In agreement with the work of Osono and Hirose (2009) the similarity between land use systems decreases as altitude increases.

The similarity between the two sites (Wondo Genet and Menagesha Suba) was higher during the dry season (*QS*= 0.126183) than during the wet season (*QS*=0.0324524) (Table 12). This result makes us to

Table 11. Showing total number of isolates from young and old host tree, commonly shared number of isolates and Sorensen similarity index.

Site and season	Age of sampling units	Total no. of isolates	No. of commonly shared isolates	*QS*
WG, Wet	Young	89	15	0.1704545
	Old	87		
WG, Dry	Young	104	19	0.1979167
	Old	88		
MS, Wet	Young	105	3	0.039278
	Old	89		
MS, Dry	Young	57	9	0.144
	Old	68		

QS: Sorensen Similarity index, WG- Wondo Genet, MS- Managesha Suba.

Table 12. number of commonly shared isolates between geographic variations and Sorensen similarity index.

Season	Sampling sites	Total no. of isolates	No. of commonly shared isolates	*QS*
Wet	Wondo Genet	176	6	0.0324324
	Menagesha Suba	194		
Dry	Wondo Genet	192	20	0.126183
	Menagesha Suba	125		

conclude that the similarity between two different geographic locations (Wondo Genet site and Menagesha Suba site) was higher during the dry season than during the wet season. The finding of this study shows that geographic location affects distribution of endophytic fungal diversity (Gore and Bucak, 2007).

Conclusion and recommendations

Variation in the type of dominant morphotaxa between ages of host within the same site, season and land use system was observed. It suggests that there occur variation in the type of dominant morphotaxa between seasons, geographic locations, age of host trees and land use systems. The diversity of endophytic fungi was numerically higher in Wondo Genet site (1600 and 2500 masl) than Menagesha Suba (2200 to 3000 masl) but statistically same.

The diversity of endophytic fungi during the wet season at both sampling sites (Wondo Genet and Menagesha) was numerically higher in trees in natural forest than trees from open land. During the dry season, numerically higher endophytic fungi diversity was recovered in open land. Seasonal variation in the diversity of endophytic fungi was recorded that is, the diversity of endophytic fungi was higher in dry season than wet season but the data is not statistically significant. Lower altitude favors higher number, unique and rarest morphotaxa than higher altitude.

Since the diversity of endophytic fungi was numerically higher in dry seasons of the year, lower altitude (Wondo Genet as in our case), and concerned bodies should act accordingly to utilize the economic significance of tree endophytic fungi. To have full information on the diversity of endophytic fungi enough samples per tree should be collected. Further researches should focus on investigation of endophytic fungi for several agro-climatic zones, at different slopes and seasons. A full-fledged taxonomic study should be pursued at a species level through polyphasic approach. Further research should be conducted on a different plant species and different organ of the plant (leaf, root, stem and seed etc). Work on antimicrobial and biocontrol properties of the endophytic fungi should be undertaken.

Conflict of interest

The author(s) have not declared any conflict of interests.

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