INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
13–284/2014/16–3–480–488
http://www.fspublishers.org

Full Length Article

Community Structure of Ectomycorrhizae Associated with *Salix* spp. Growing in Two Different Climatic Regions of Pakistan

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Abstract

Ectomycorrhiza is a mutualistic symbiotic relationship between the roots of higher plants and fungi. *Salix* is an important host genus for ectomycorrhizal symbiosis growing in a variety of environmental conditions. Eleven different types of ectomycorrhizal morphotypes were isolated from different species of *Salix* growing in two geologically distant zones of Pakistan with varied environmental conditions. KPK zone has moist temperate while Lahore zone falls in semi-arid climate. These morphotypes were characterized on morpho-anatomic basis. Out of these eight belonged to KPK zone and three to Lahore zone. Three morphotypes were previously undescribed and six have been found for the first time associated with *Salix* spp. Two are new records for Pakistan. Species richness and diversity was found higher in KPK zone as compared to Lahore zone where species richness and diversity was lower but a high number of ectomycorrhizal morphotypes were found in comparison with KPK zone. © 2014 Friends Science Publishers

Keywords: Ectomycorrhizas; Salicaceae; Morphotypes; Ecological zones; Community

Introduction

Belonging to the family Salicaceae, the genus *Salix* L. comprises about 450 species worldwide distributed mostly in the Northern Hemisphere (Argus, 1997). These species are characterized by particular physiological adaptations and ecological flexibility which are predisposed to use in conservation and environmental projects in many climatic zones and adverse environmental conditions. The economic importance of *Salix* is currently increasing and emerging in a wide array of practical applications to restore damaged ecosystems. These tree species are widely used in wetlands and riparian situations (Yulia and Martin, 2004).

Among the 3 genera, Salicaceae is represented by 2 in Pakistan with 32 species (Ali, 2001). Being cosmopolitan in distribution, it is found naturally in different climatic regions of Pakistan, sometimes cultivated on arable sites. From Lahore it is represented by two species viz., *Salix babylonica* and *S. tetrasperma*. Many species are present abundantly in Himalayan region of Pakistan besides of *S. babylonica* and *S. tetrasperma*, these include *S. alba, S. herbacea , S. iliensis, S. denticulate, S. persica* and *S. wilhelmsiana* (Qureshi *et al*., 2007).

Trappe (1962) reported that many species of the Salicaceae form ectomycorrhizae (ECM). Katenin (1964) examined members of the genus *Salix* in the tundra of the U.S.S.R. and found ectomycorrhizae associated with all species. Stutz (1972) reported *S. arctica* Pallas to be mycorrhizal, and Miller *et al.* (1974) reported all four species of *Salix* present at Barrow, Alaska, to be ectomycorrhizal. From Pakistan, *S. alba* is reported as ectomycorrhizal host for *Suilus cibiricus* (Sarwar *et al*., 2011).

Most of the studies in Pakistan depend on the counts of fungal sporocarps rather than assessing the ectomycorrhizal symbionts actually colonizing roots of trees (Jabeen *et al.,* 2012). On the other hand, morphotyping uses macroscopic and microscopic characteristics to define individual fungal morphotype allowing for a thorough description of each morphotype so that they can be distinguished from others (Dahlberg, 2001). Up till now, 52 ECM morphotypes published in identified and unidentified form have been reported from Pakistan (Khalid and Niazi, 2003; Kazmi *et al*., 2004; Niazi *et al*., 2006, 2007, 2009, 2010; Sarwar *et al*., 2011; Hanif *et al*., 2012; Jabeen *et al*., 2012; Sarwar *et al*., 2012; Ashraf *et al*., 2012). The objective of this work is to find out diversity of ECM associated with *Salix* spp. growing in two different climatic regions of Pakistan which differ on the basis of soil properties, temperature and rain fall patterns, as well as to give a comprehensive information about the below ground community structure of ECM fungi. Community diversity is mainly based upon species richness, the number of species in the community and community evenness, a measure of the abundance of each species in the community. In most ECM communities, with a few common species and a large number of rare species greatly influence the assessment of species richness. From species accumulation curve, it becomes possible to overcome this problem. The present work focuses on the comparison of richness diversity and

To cite this paper: Jabeen, S. and A.N. Khalid, 2014. Community structure of ectomycorrhizae associated with *Salix* spp. growing in two different climatic regions of Pakistan. *Int. J. Agric. Biol*., 16: 480–488

abundance of ECM morphotypes of two zones and judge the factors influencing the differences between the parameters of community structure.

Materials and Methods

Sampling Site Description

Lahore, capital of Punjab province (Lahore zone), at 217 meters above sea level (m.a.s.l.) features a hot semi-arid climate with rainy, long and extremely hot summers, dry cold winters, a monsoon and dust storms. The weather of Lahore is extreme at 40-48°C during the months of May and June. From late June, the monsoon seasons starts, with heavy rainfall till August with an average 470 mm with and 30% humidity. On the other hand, moist temperate regions (Khyber Pakhtunkhwa/KPK zone) extended from Muree hills, Ayubia, Nathiagali to Miandam from1890 to 2500 m. a.s.l. between subtropical pine forests and sub alpine forest. The weather is extreme cold in December and January at 3°C while it reaches up to 26°C during summers. Average rain fall of the area is 700 mm with 57% humidity (Ahmed *et al*., 2006) (Table 1).

Sampling of Morphotypes

Sampling sites were visited a number of times from October 2009 to September 2010. Mature trees with a trunk diameter of 50–60 cm were selected. From each zone ten trees were selected and soil blocks of 15 cm^3 were excavated using shovel/spade at 15 cm to 1 m from the trunk of each tree. Two replicate samples per tree were collected and brought to the laboratory in polythene bags and catalogued.

Isolation of Roots, Morphotyping and Storage of ECM

The soil blocks were soaked in tap water for overnight to loosen the adhering soil particles then shifted to 2 mm sieve and placed under shower to remove the soil. The ectomycorrhizae were carefully sorted into morphotypes according to their morphological features. Criteria for sorting included ramification, colour, size, associated hyphae, rhizomorphs, cystidia etc. The attached soil particles were removed with the help of fine squirrel hair brush under Meiji Techno stereoscope. Individual root tips were dissected and stored according to their characteristics. Only mycorrhizal root tips with the same morphology, from one sample were joined resulting in a given morphotypes sample. Washed roots were kept in McCartney bottles after two to three washings in two drops of Max liquid for comparative morpho-anatomic studies.

Photography of ECM

Morphotypes were placed in clean petriplate containing water with contrasting background below the petriplate in order to clearly visualize and distinguish the color of ECM morphotypes and focused under Meji Techno Stereoscope equipped with Canon Power Shot G10 14.7 megapixel camera.

Microscopic Characterization of Morphotypes

Separations made under dissecting microscope were confirmed by examining in more detail under compound microscope. Morphotypes were anatomically characterized following Agerer's methodology. Morphological characteristics were examined under stereomicroscope. Clear view of mantle was obtained by peeling off mantle from a tip and mounting its outer and inner surface on slides in lactic acid as mounting medium and observed in detail, hyphae or hyphal cells. The internal structures of rhizomorphs, emanating hyphae, and cystidia were also observed and measured using micro meter and drawn with the help of Camera Lucida. Characteristics of mantle, emanating hyphae, rhizomorphs and cystidia type were used to characterize definitely recognizable mycorrhizal types and to relate these to different species of fungal symbionts for taxonomic purpose.

Species Richness Curve

Species richness is the number of different species represented in an ecological region. Species richness of the two ecological zones was estimated by species richness curve drawn using Estimate S software.

Simpson's Diversity Index

To quantify the ectomycorrhizal diversity, Simpson's Diversity Index was implied. The term 'Simpson's Diversity Index' refers to any one of 3 closely related indices. In this study, Simpson's Index (D) was used. Where: $D = \Sigma n (n-1) / N (N-1)$ and n= total number of individuals of a particular species and N=total number of individuals of all species.

The value of D ranges between 0 and 1. Index, 0 represents infinite diversity and 1, no diversity. That is, the bigger the value of D, the lower the diversity.

Results

ECM of *Salix* spp. from Lahore Zone

Out of 98 root tips isolated from genus *Salix,* 54 were found ectomycorrhizal; and 28 were found in association with *S. babylonica*. Among these 12 belong to *Populirhiza lahorensis*, 9 to *Quercirhiza tomentellocystidiata* and 7 to *Lactarious deliciosus* with percent abundance of 42.85, 32.14 and 25, respectively.

Morphotypes isolated from *S. tetrasperma* were 26. Out of these, 19 belong to *Quercirhiza tomentellocystidiata* and 7 to *Populirhiza lahorensis* with percent abundance of 82.60 and 17.39, respectively (Fig. 3).

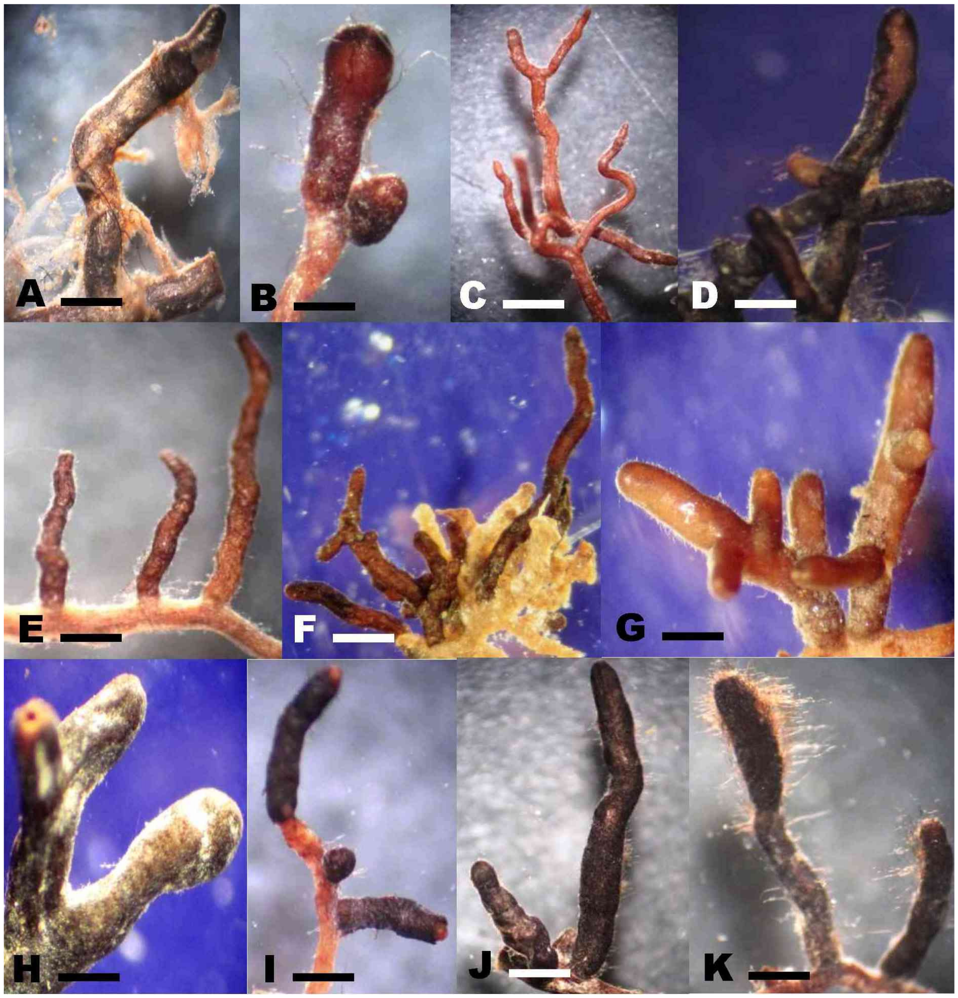

Plate 1: Morphological features of ectomycorrhizal morphotypes (A) *Dermocybe cinnamomea* (B) *Genea hispidula* (C) *Lactarius deliciosus* (D) *Piceirhiza obscura* (E) *Populirhiza lahorensis* (F) *Quercirrhiza tomentellocystidiata* (G) *Russula foetens* (H) *Salicirhiza gigantica* (I) *Salicirhiza khanspurensis* (J) *Salicirhiza pakistanica* (K) *Tuber aestivum*. Scale bar for A: 2 mm, B: 1.2 mm, C: 3.6 mm, D: 2 mm, E, F and G: 1.2 mm, H: 8 mm, I: 1.2 mm, J: 1.6 mm, K: 2.4 mm

Ectomycorrhizae formed by *Lactarius deliciosus* L. with root system of *S. babylonica* have been identified (Plate 1C, Plate 2R-T). Previously they have been reported with *Pinus wallichana* (Agerer, 1987-2006). They are first time reported with *S. babylonica*.

Ectomycorrhizal morphotypes of *Populirhiza lahorensis* have been found in association with *S. tetrasperma* (Plate 1E; Plate 2Y-a). Previously these were reported from *Populus euramericana* (Jabeen *et al*., 2012).

Morphotypes of *Quercirhiza tomentellocystidiata* have been found with *S. babylonica* (Plate 1F; Plate 2b-e). Previously it has been reported on *Quercus suber* (Agerer, 1987-2002) and *P. euramericana* (Jabeen *et al.,* 2012).

ECM of *Salix* spp. from KPK Zone

From KPK zone total 99 root tips were isolated from *Salix,* 85 have been found mycorrhizal. Among these, 38 were found associated with *S. babylonica*, 14 belong to *Dermocybe cinnamomea*, 8 to *Picirhiza obscura,* 6 to *Genea hispidula* and 4 to *Salicirhiza pakistanica* with percent abundance of 43.75, 25, 18.75 and 12.5 respectively. Morphotypes associated with *S. tetrasperma* were 25. Among these, 15 belong to *Russula foetens*, 5 to *S. gigantica* and 5 to *S. pakistanica* with percent abundance of 60, 20 and 20 respectively. Morphotypes associated with *S. herbacea* were 20. Among these 15 belong to *D. cinnamomea* with 25% abundance and 5 to *S. khanspurensis* with 75% abundance. Morphotypes isolated from *Salix* sp. were only 4 which belong to *Tuber aestivum* (100% abundance) (Fig. 4).

Ectomycorrhizal symbiosis of *D. cinnamomea* having hairy mycorrhizal system in the form of hyphal fans previously reported with *Picea abies* (L.) Karst. (Agerer, 1987-2006) (Plate 1A; Plate 2L-N). It is first time being reported with *S. herbacea* in this investigation.

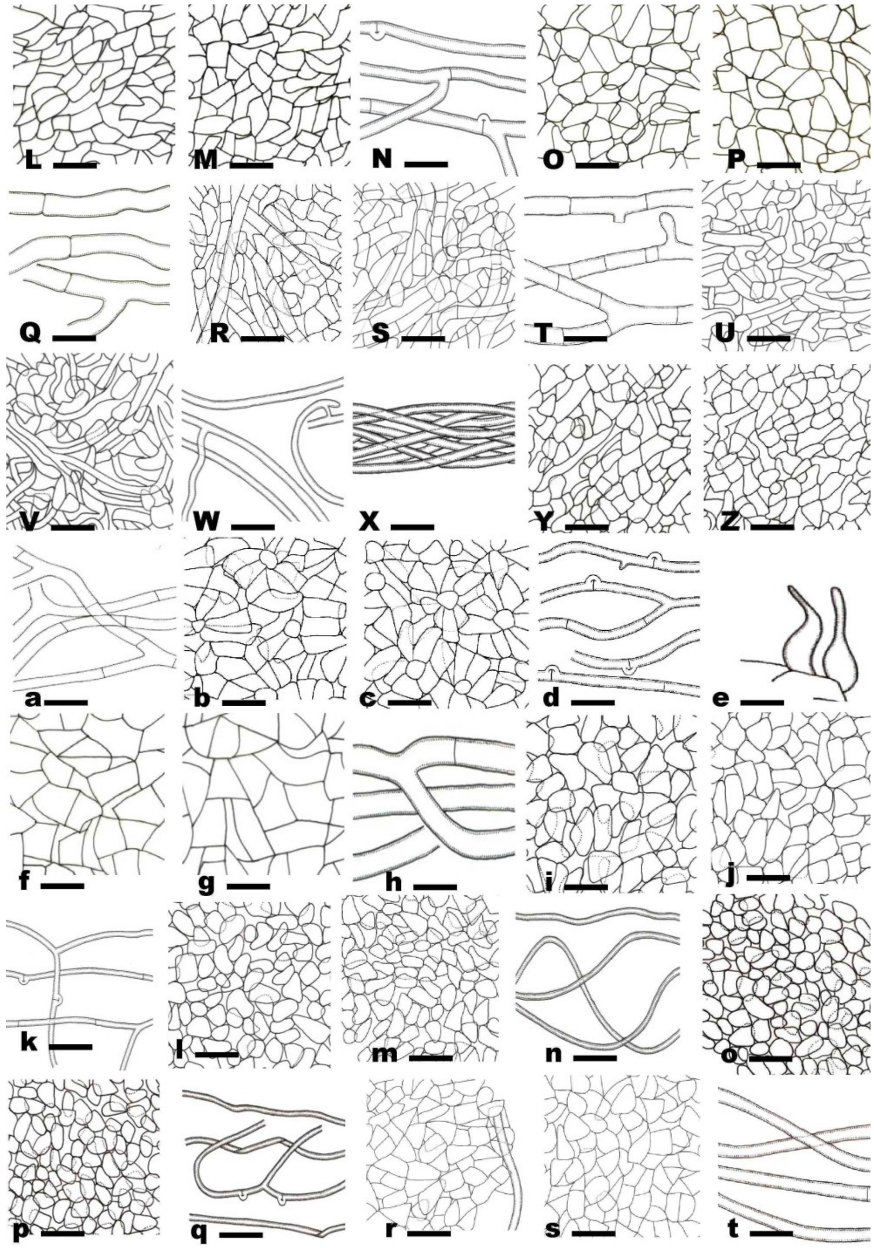

Plate 2: Anatomical features of ectomycorrhizal morphotypes (L) Outer Mantle (OM) (M) Inner Mantle (IM) (N) Emanating Hyphae (EH) of *Dermocybe cinnamomea*; (O) OM (P) IM (Q) EH of *Genea hispidula*; (R) OM (S) IM (T) EH of *Lactarius deliciosus*; (U) OM (V) IM (W) EH (X) Rhizomorphs of *Piceirhiza obscura*; (Y) OM (Z) IM (a) EH of *Populirhiza lahorensis*; (b) OM (c) IM (d) EH (e) Cystidia of *Quercirrhiza tomentellocystidiata*; (f) OM (g) IM (h) EH of *Russula foetens*; (i) OM (j) IM (k) EH of *Salicirhiza gigantica*; (l) OM (m) IM (n) EH of *S. khanspurensis*; (o) OM (p) IM (q) EH of *S. pakistanica*; (r) OM (s) IM (t) EH of *Tuber aestivum*. Scale bar for L, M and N: 15 µm O, P and Q: 20 µm R and S: 22.5 µm T: 20 µm U and V: 15 µm W and X: 20 µm Y, Z and a: 17.5 µm b: 40 µm c: 45 µm d: 47.5 µm e: 1.75 µm f and g: 27.5 µm h: 12.5 µm I and j: 15 µm k: 45 µm l, m, n, o and p: 25 µm q: 40 µm r, s and t: 15 µm

Ectomycorrhizal morphotypes of *G. hispidula* Berk. et. Br. isolated from *S. babylonica* are characterized on the basis of simple or irregularly pinnate type of ramification. Anatomically they possess pseudoparenchymatous mantle layers with angular cells throughout and emanating hyphae with wide diameter and simple septa (Plate 1B; Plate 2O-Q). This fungus is first time reported with *S. babylonica* and morphotype of this fungus is a new record to the mycoflora of Pakistan.

P. obscura associated with *S. babylonica* shows similarities with that ECM morphotypes of *P. abies* (Plate 1D; Plate 2U-X). The fungus is previously reported with *P.*

abies (Agerer, 1987-2006) and has a wide host range. So this is first time being reported with *S. babylonica*.

R. foetens associated with *S. tetrasperma* has been reported with *P. alba* (Agerer, 1987-2002) and *P. ciliata* (Jabeen *et al*., 2012) (Plate 1G; Plate 2f-h). On the other hand, morphotypes of *S. gigantica* isolated from *S. tetrasperma* has been characterized on the basis of its large dichotomous to coralloid ectomycorrhizal system and pseudoparenchymatous mantle layers with hyphae variable in size and shape. Emanating hyphae branched with clamped septa (Plate 1H; Plate 2i-k). It does not show any characteristic resemblance with any of the reported taxon and seems previously undescribed.

Another ectomycorrhizal morphotype *S. khanspurensis* isolated from *S. herbacea* is previously undescribed and is characterized by its black, simple, smooth to grainy morphotype with infrequently formed emanating hyphae on ectomycorrhizal tips. Mantle cells are pseudoparenchymatous with angular layers to roundish cells, emanating hyphae with narrow diameter and without septa. Morphotypes of these two taxa can be compared with that of *P. hyphaeata* (Jabeen *et al*., 2012). Differences between these two are the presence of abundant emanating hyphae in *P. hyphaeata* and infrequent in *S. khanspurensis*. Anatomically, they differ in inner mantle hyphae, which are plectenchymatous in *P. hyphaeata* and pseudoparenchymatous in *S. khanspurensis*. Emanating hyphae with septa are present in *P. hyphaeata* but are absent in *S. khanspurensis* (Plate 1I; Plate 2l-n).

Morphotypes of *S. pakistanica* are characterized by its dark brown to black, simple irregular system with somewhat beaded appearance. Inflated emanating hyphae emerging from the mantle layer distinguish it from other described taxa (Plate 1J; Plate 2o-q).

Ectomycorrhizal system of *T. aestivum* isolated from *Salix* sp. has been characterized on the basis of wooly appearance due to abundance of long wooly cystidia present on the tip of mycorrhizal morphotype and pseudoparenchymatous mantle with angular cells. These morphotypes can be compared with mycorrhiza of *T. aestivum* associated with *Corylus avellana* L. (Agerer, 1987-2006). Wooly appearance and pseudoparenchymatous mantle layers are similarities, but the main difference is the color of ectomycorrhizal morphotypes which is reddish brown in morphotypes isolated from *C. avellana* and dark brown to black in morphotypes associated with the root system of *Salix* sp. These differences might be due to maturity of ectomycorrhiza or due to difference in host species (Plate 1K; Plate 2r-t).

Total eleven different types of ectomycorrhizal morphotypes were isolated and characterized. From Lahore zone, 3 ectomycorrhizal fungal taxa viz., *L. deliceosus, P. lahorensis and Q. tomentellocystidiata* were isolated. From KPK zone 8 fungal taxa were isolated. Which includes, *D. cinnamomea, G. hispidula, P. obscura, R. foetens, S. gigantica, S. khanspurensis, S. pakistanica* and *T. aestivum.*

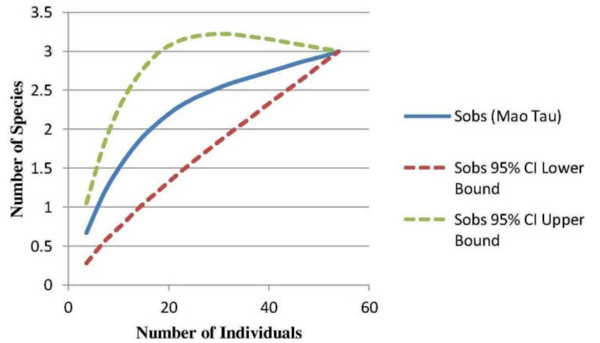

Fig. 1: Species accumulation curve (Lahore zone)

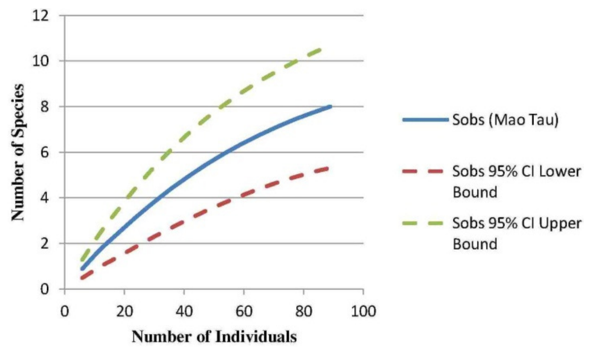

Fig. 2: Species accumulation curve (KPK zone)

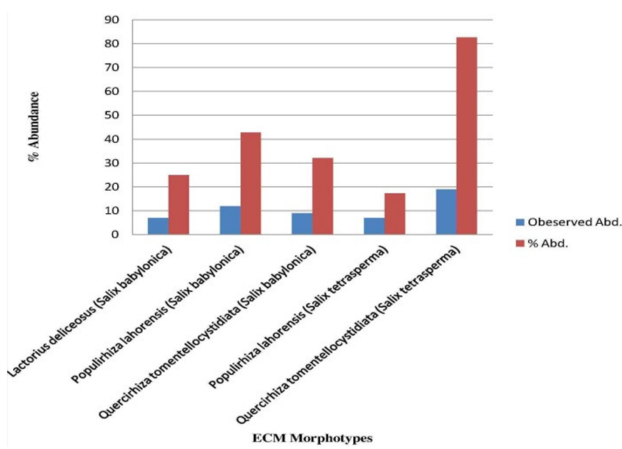

Fig. 3: Percentage abundance of ectomycorrhizal morphotypes in Lahore zone

Among these, 3 morphotypes were previously undescribed and given tentative names (*S. gigantica*, *S. khanspurensis* and *S. pakistanica*) and six have been found for the first time associated with Salix spp. (*D. cinnamomea, G. hispidula, P. obscura, S. gigantica, S. khanspurensis and S. pakistanica*). Two morphotypes of fungal species are new records for Pakistan (*D. cinamomea* and *G. hispidula*).

Species Accumulation Curves and Estimation of Species Richness

The number of ECM morphotypes observed from Lahore

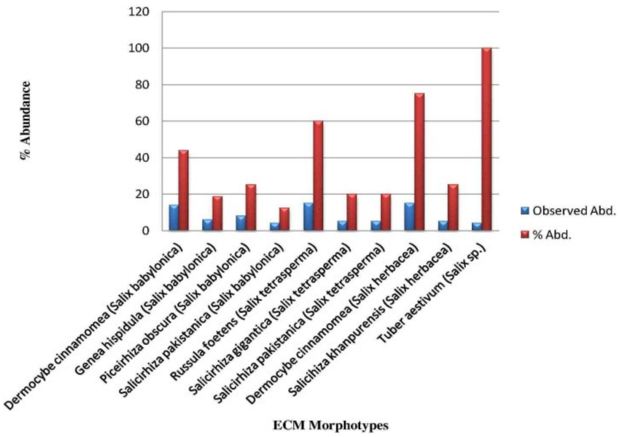

Fig. 4: Percentage abundance of ectomycorrhizal morphotypes in KPK zone

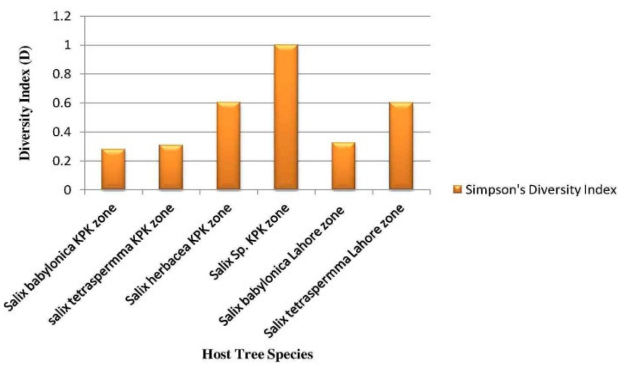

Fig. 5: Simpson's diversity indices of ectomycorrhizal morphotypes associated with different *Salix* spp. in both zones

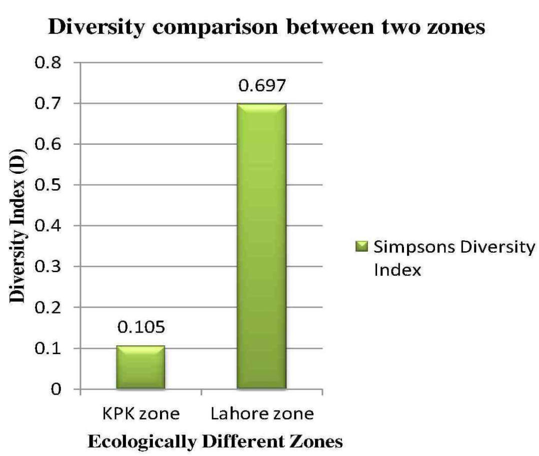

Fig. 6: Ectomycorrhizal diversity comparison between Lahore zone and KPK zone

zone and KPK zone were 3 and 8, respectively. Total number of species from these zones was estimated from 'collectors curve', a graph in the form of solid line between the number of species versus number of individuals. The smooth line of the graph can be obtained using Estimate S to get infinite number of randomizations using an algorithm developed by Mao Chang Xuan [Sobs (Mao Tau)] allowing to calculate the confidence interval.

Among three lines in the graph, the middle one is very close to the original Sobs (Species observed) curve. The other two lines are the confidence limits. The species accumulation curve looks as though our coverage of species is still rather incomplete (Fig. 1 and 2).

Species Richness Comparison between Lahore Zone and KPK Zone

For the comparison of two sites inferences has been made on the basis of interplotation. In Lahore zone 54 individual mycorrhizal root tips were isolated from 3 ectomycorrhizal morphotype/fungal species. The number of species expected from KPK zone can be estimated from this zone, using species accumulation curves (Fig. 1 and 2).

With 15 samples from KPK Zone, 53.4 individual ectomycorrhizal root tips would be isolated, which is the nearest value to 54. The average number of species recorded would be 5.93 [Sobs (Mao Tao)]. This is more than that of the 3 species recorded from the Lahore zone with a very similar number of individual ectomycorrhizal root tips, so it appears that KPK zone is indeed richer in ectomycorrhizal fungal species as compared to Lahore zone.

Percentage Abundance and Diversity Comparison of Ectomycorrhizae from Both Zones

In the present study diversity of ECM fungi associated with *Salix* spp. growing in two different ecological zones (which differ in climatic conditions e.g., temperature, rainfall, soil conditions, humidity etc.) has been studied by using morpho-anatomical characterization. In Lahore zone *Q. tomentellocystidiata* showed high percentage abundance in association with *S. tetrasperma* (82.60%). Whereas *P. lahorensis* showed high abundance with *S. babylonica* (42.85%). Other taxa showed low abundance (Fig. 3).

In KPK *D. cinnamomea* exhibited highest percentage abundance in association with *S. herbacea* and *S. babylonica* (75% and 43.75, respectively), while *R. foetens* with maximum abundance (60%) found in association with *S. tetrasperma.* Only *T. aestivium* revealed from *Salix* sp. There is no comparison for its abundance (Fig. 4).

Simpson's diversity index measures the diversity taken place into account the number of species present as well as relative abundance of each species from both zones. Among host tree species, high ECM diversity was found in association with *S. babylonica* from KPK zone, while lowest diversity was observed with *Salix* sp. from KPK zone with highest diversity index (Fig. 5). High

Table 1: Climate, physiography, edaphic conditions and physiognomy of two climatic regions

Site Description		Lahore (Punjab) zone	KPK zone
Climate	Climatic Region	Semi Arid (Sethi, 2003)	Moist Temperate (Sethi, 2003)
	Temperature (Mean Annual)	26.5°C (Malik and Sukhera, 2012)	10°C (Malik and Sukhera, 2012)
	Rain fall (Mean Annual)	700 mm (Ahmed *et al*., 2006)	1200mm (Ahmed *et al*., 2006)
	Humidity	30% (Ahmed *et al*., 2006)	57% (Ahmed *et al*., 2006)
Physiography	Geography	Most of the area is a level plain formed by the Indus and its tributaries (Khalid and Niazi, 2003)	The area ranges from lofty mountains with rugged valleys to undulating and dissected sub-mountain plateaus and flat mountains (Khalid and Niazi, 2003)
	Elevation (Hight Above Sea Level)	217 m (Government of the Punjab, 2007)	3000-4000 m (Khan, 1999)
Edaphic characters	Soil	Silty clay, loamy clayey (Shafique *et al*., 2012)	Rock out crop, Silt loam to silty clays in valleys, non-calcareous to slightly calcarious (Khan, 2004)
	pH	8.58 (Saline-sodic) (Akhtar *et al*., 2004)	< 7 (Acidic due to high organic matter) (Irshad and Khan, 2012)
	Organic Matter	<1% (Fatima *et al*., 2012)	3-4% (Irshad and Khan, 2012)
Physiognomy	Vegetation Ecological Zone	Sub-tropical thorn forest	Moist temperate pine forest
	Some dominant tree genera	*Accacia, Albezia, Cassia, Dalbergia, Eucalyptus, Melia, Morus, Populus, Salix, Zizyphus* (Sheikh, 1993)	*Abies, Alnus, Cedrus, Picea, Pinus, Populus, Quercus, Salix, Taxus* (Sheikh, 1993)

Table 2: Pearson correlation coefficients between morphotypes and environmental conditions

Characters		DI	Abd	pH	Temp	Ele	OM	RF
DI	Pearson Correlation	1	.831**	.505	.505	-.505	-.505	-.505
	Sig. (2-tailed)		.002	.113	.113	.113	.113	.113
	N	11	11	11	11	11	11	11
Abd	Pearson Correlation	.831**	1	.619*	.619*	-.619*	-.619*	-.619*
	Sig. (2-tailed)	.002		.042	.042	.042	.042	.042
	N	11	11	11	11	11	11	11
pH	Pearson Correlation	.505	.619*	1	1.000**	-1.000**	-1.000**	-1.000**
	Sig. (2-tailed)	.113	.042		.000	.000	.000	.000
	N	11	11	11	11	11	11	11
Temp	Pearson Correlation	.505	.619*	1.000**	1	-1.000**	-1.000**	-1.000**
	Sig. (2-tailed)	.113	.042	.000		.000	.000	.000
	N	11	11	11	11	11	11	11
Ele	Pearson Correlation	-.505	-.619*	-1.000**	-1.000**	1	1.000**	1.000**
	Sig. (2-tailed)	.113	.042	.000	.000		.000	.000
	N	11	11	11	11	11	11	11
OM	Pearson Correlation	-.505	-.619*	-1.000**	-1.000**	1.000**	1	1.000**
	Sig. (2-tailed)	.113	.042	.000	.000	.000		.000
	N	11	11	11	11	11	11	11
RF	Pearson Correlation	-.505	-.619*	-1.000**	-1.000**	1.000**	1.000**	1
	Sig. (2-tailed)	.113	.042	.000	.000	.000	.000	
	N	11	11	11	11	11	11	11

**. Correlation is significant at the 0.01 level
*. Correlation is significant at the 0.05 level
DI: Diversity Index, Abd: % Abundance, Temp: Temperature, Ele: Elevation, OM: Organic Matter, RF: Rain Fall, N: Number of fungal taxa

ECM morphotype diversity was observed with trees found in KPK zone (0.105), while *Salix* spp. of Lahore zone showed low ECM diversity with their root system (0.697) (Fig. 6).

Discussion

Below ground fungal population is a critical field of study to understanding why certain ectomycorrhizal (ECM) fungi dominate in certain forests? In this investigation, 11 different fungal taxa were isolated from 30 root samples of *Salix* spp. taken from two different, geologically distant, and climatically varied areas of Pakistan. Out of these, eight (8) taxa belong to KPK zone and three (3) to Lahore zone. Among these, four (4) morphotypes are previously undescribed and seven (7) have been found for the first time associated with *Salix* spp. Four (4) morphotypes of fungal species are new records for Pakistan.

It appears that in Lahore zone, morphotypes of ECM fungal species are abundant but as compared to KPK zone. As species richness increases, diversity also increases. Diversity index shows that in KPK zone, ECM diversity is greater in KPK zone, while lower in Lahore zone. As far as species richness is concerned, it was estimated from species richness curve drawn using Estimate S, KPK zone is richer in number of ECM species as compared to Lahore zone. The probability of the species finding is higher in KPK zone in comparison with Lahore zone.

Although the host genera are same but difference in their ecology is the main factor influencing the abundance, diversity and richness of ECM. This difference is due to the difference in climate, physiography, physiognomy and edaphic conditions of these two zones (Table 1). Percentage abundance and the value of diversty index are positively correlated with pH and temperature, while a negative correlation was recorded with altitude and amount of rain fall as well as organic matter content (Table 2). In KPK, climate is humid with high organic content in soil provides mycobiont the acidic conditions which support the growth of fungal fruit bodies and mycorrhizae. While the semi-arid and saline soil conditions in Lahore limits the ECM diversity associated with *Salix* spp. Fungi that do not form obvious fruiting structures form the major mycorrhizal abundance (Gardes and Brun, 1996; Jonsson *et al*., 1999).

In conclusion, the study demonstrated that ecological factors strongly influence the taxonomic diversity of ECM fungi. Altitude, temperature, rainfall and soil conditions greatly affect the type of ectomycorrhizae. Some fungi can tolerate high temperature and saline soil conditions as in Lahore zone where the root tip concentration is higher. The results suggest that rare species are relatively less competitive in harsh climatic conditions, and increasing dominance reduces overall diversity. The ECM diversity in KPK zone with low temperature, high rain fall pattern and acidic soil conditions are the parameters making the diversity two folds higher than in Lahore zone where abundance of morphotypes are found belonging to only three fungal species from equal number of root samples taken from the rhizosphere of *Salix* spp. of both these zones. Körner (2007) reported that altitude is the main factor determining ECM fungal richness. Among the environmental variables, the mean annual temperature had the strongest effect on richness and was most strongly correlated with altitude, which is a common phenomenon.

Generally, ECM fungi are important to plant when available nutrients in the soil are limiting, but the diversity decrease when nutrients are readily available (Jones and Smith, 2004). Despite these variables, host tree species also had strong effect on ECM community composition. Dominant tree species in the area differentially modify soil properties and nutrient availability (Tedersoo *et al*., 2008a, b). The soil environment is one of the most complex parts of the forest ecosystem. ECM ecology is still largely focused on identifying species and describing fungal habitat parameters, such as forest stand age and availability of nutrients.

Although this information is rapidly accumulating, as is understating of mycorrhizal functions. Nevertheless, this review reveals that many gaps in knowledge remain to be filled, and mycorrhizologists should focus on little known geographic areas, ecosystems, host trees and fungal groups in future studies in order to face the challenges, the ectomycorrhizal community possess. With more advances in these areas, forest management may be improved so that it sustains a healthy below-ground environment.

Acknowledgements

We thank Dr. Abdul Rehman Khan Niazi and Dr. Muhammad Hanif for assistance in field work. We are also grateful to Ms. Sobia Ilyas, Mr. Abdul Razaq and Ms. Nousheen Yousaf for their suggestions in data compilation. Thanks is to Ms. Aneela Yasmeen for technical support.

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(Received 01 March 2013; Accepted 27 May 2013)