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RESEARCH ARTICLE

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Biodiversity study of endophytic fungi associated with two *Quercus* species in Iran

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Abstract

Aim of study: In this study, frequency and diversity of fungal endophyte communities inhabiting twigs and branches of apparently healthy *Q. macranthera* and *Q. brantii* in East Azerbaijan and Lorestan provinces of Iran is presented.

Area of study: East Azerbaijan and Lorestan provinces in Iran.

Materials and methods: Culturable fungal endophytes were recovered from wood tissues using routine technique for isolation of fungal endophytes. The identity of fungal isolates were determined based on morphological characteristics and sequences data of ITS-rDNA region and *Beta-tubulin* gene. Frequency and diversity among fungal communities were analyzed using chi-square test and biodiversity indices.

Main results: The highest frequency and diversity was detected for fungal endophyte community recovered from *Q. macranthera* and East Azerbaijan province. The assemblage of endophytic fungi characterized in this study in healthy tissues of oak trees indicates that some of the fungi are possible latent pathogens such as *Biscogniauxia mediterranea* with 18.28% frequency followed by *Alternaria alternata* and *Trichothecium roseum* respectively. Two fungal taxa of *Pyronema domesticum* and *Valsa persoonii* are reported for the first time in Iran. Overall, the results of this study show that the plant species and growth location influence frequency and diversity of culturable fungal endophytic communities of *Quercus* in Iran.

Additional keywords: *Quercus macranthera, Quercus brantii,* Fungal endophytes, Molecular identification.

Abbreviations used: CBS (Centraal Bureau voor Schimmelcultures); CCTU (Culture Collection of University of Tabriz); GTR (General Time Reversible); HKY (Hasegawa Kishino Yano); ITS-rDNA (Internal Transcribed Space); km (kilometer) ; PDA (Potato Dextrose Agar); TUB (Tubulin).

Authors' contributions: Saeid Ghasemi-Esfahlan and Sima Khodaei were responsible for the isolation, sampling and participated in the writing of the manuscript. Kaivan Karimi conducted the analyses together with Saeid ghasemi-Esfahlan. Majid Tavakoli participated in sampling. Ilaria Pertot and Mahdi Arzanlou participated in the writing of the manuscript and supervision. All authors have read and approved the final manuscript.

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Introduction

Certain microscopic fungi live at least a part of their life cycle inside the tissues of the plants without causing visible signs or symptoms and, therefore, are named endophytes (Petrini, 1996). Fungal endophytes are a taxonomically and ecologically heterogeneous group and seem to make up a large fraction of the fungal biodiversity (Petrini *et al*., 1992; Saikkonen *et al*., 1998; Arnold *et al*. 2000, 2003). Endophytes in plants can play important ecological roles, e.g. mediating plant defense reactions against pathogens and herbivores or influencing host responses to abiotic stressors such as drought (Costa Pinto *et al*., 2000; Arnold *et al*., 2003; Schardl *et al*., 2004; Arnold & Engelbrecht, 2007; Mejia *et al*., 2008; Estrada *et al*., 2013). However, some endophytic fungi have proven to be latent pathogens of plant hosts. Furthermore, the role of some endophytes in host plants is still unclear (Mirabolfathy, 2013).

Previous studies have shown that the diversity, abundance, and species composition of endophytic fungi can be highly affected by the locality in which a specific plant occurs (Carroll & Carroll, 1978; Petrini *et al*., 1982; Bills & Polishook, 1992; Fisher *et al*., 1994; Hata & Futai, 1996; Bayman *et al*., 1998; Arnold, 2001; Higgins *et al*., 2007). At larger geographical scales, diversity of endophytic fungi varies due to latitude and annual rainfall (Arnold & Lutzoni, 2007), although the impact of co-varying factors, such as plant diversity, remains to be studied. Similarly, due to history of land use, plantation, and other factors, species diversity of endophytes differs at small scales (Gamboa & Bayman, 2001). Furthermore, the endophyte composition differs because of localities (Fisher *et al*., 1995; Frohlich & Hyde, 1999; Arnold *et al*., 2003). For example, Arnold *et al*. (2003) reported a distinctive endophytic composition associated with *Theobroma cacao* at different sites in Panama. Many studies investigating host associations of endophytic fungi have focused on distantly related plants, which grow within the same geographic areas. Contradictory results, however, have been reported about the predominance of host specificity (Sieber 1989; Suryanarayanan & Kumaresan 2000; 2005; Arnold *et al*., 2000; Cannon & Simmons, 2002; Mohali *et al*., 2005; Higgins *et al*., 2007).

Quercus macranthera Fisch. & C.A. Mey ex Hohen (black oak) and *Q. brantii* Lindl. which are the most common plant species in Iran have never been investigated before in term of composition of cultivable fungal endophytic populations. Therefore, the aim of this research was to characterize fungal endophytic communities of barks in *Q. macranthera* and *Q. brantii* and understand if the plant host species or the geographical sites of growth are responsible for shaping the culturable fungal endophytic populations.

Materials and Methods

Sampling

The culturable endophytic species of oak trees in Arasbaran protected area (Hatam-baig and Kaleibar regions, located in East Azerbaijan province), northwestern Iran, as well as oak forests of Zagros region (Veisian, Shurab, Kaka Sharaf, Khorramabad and Chegani counties located in Lorestan province), west of Iran, were identified based on molecular characteristics (Fig. S1 [suppl.]). For this purpose, bark samples from 83 apparently healthy oak trees (one sample from each plant at the chest height and from the same side of the trunk at the height of about 1.5 meters) were randomly collected in these regions between June and September 2014. Distance between sampling sites (km) is shown in Table S1 [suppl.].

Endophytic fungi isolation

Culturable endophytes were isolated following the procedure described by Helander *et al*. (2007) with some modifications (Blumenstein, 2010). Briefly, approximately 3 cm-long pieces from apparently healthy and living parts of each bark (cork cambium (phellogen) and phelloderm) sample were cut, surface sterilized using 75% ethanol, 4% Na-hypochlorite solution and 75% ethanol, for 30 seconds, 5 minutes and 15 seconds, respectively. The sterilized material was air dried for 5 minutes, cut in smaller pieces (approximately 5 × 5 mm^2) and plated in Petri dishes containing potato dextrose agar (PDA; Merck, Germany). The Petri dishes were then incubated at room temperature in dark and inspected daily for two weeks for fungal growth. Pure cultures were established using a single spore method or hyphal tip technique. The identity of fungal strains was determined in genus level primarily based on morphological characteristics (Sutton, 1980; Seifert *et al*., 2011) and then further confirmed by DNA phylogenetic analyses. The cultures were deposited in the living Culture Collection of University of Tabriz (CCUT), Tabriz, Iran.

DNA phylogeny

Total genomic DNA was extracted from fresh fungal mycelia following the protocol of Möller *et al*. (1992). The primer pairs ITS1/ITS4 (White *et al*., 1990) and Bt2a/Bt2b (Glass and Donaldson, 1995) were used to amplify ITS-rDNA and partial Beta-tubulin gene (TUB), respectively. The reaction mixture and thermal cycling condition were the same as described by Arzanlou and Khodaei (2012) and Karimi *et al*. (2016). PCR products were sequenced in both directions using a BigDye Terminator v. 3.1 cycle sequencing kit (Applied Biosystems, USA) as recommended by vendor and analyzed on an ABI Prism 3700 (Applied Biosystems).

Raw sequence files were edited manually using SeqManII (DNASTAR Inc., USA) and a consensus sequence was generated for each sequence. Sequences were subjected to Blast search analysis against the NCBI's GenBank sequence database using Megablast for sequence similarity. Sequences with high degrees of similarity and ex-type strains correspond to each taxon obtained in this study were downloaded. For each locus, the sequences obtained from GenBank together with sequences generated in this study were aligned

using the multiple sequence alignment online interface MAFFT (Katoh & Toh, 2008) and, if necessary, adjusted manually in MEGA v. 6 (Tamura *et al*., 2013). The best evolutionary model for each data partition was selected using the software MrModelTest v. 2.3 (Nylander, 2004). For phylogenetic analysis, bayesian inference (BI) was performed with MrBayes v. 3.2.1 (Ronquist & Huelsenbeck, 2003). The resulting phylogenetic tree was printed using Fig Tree ver. 1.3.1 (http://tree.bio.ed.ac.uk/software/figtree/) (Rambaut, 2009). Sequences derived from this study were deposited in NCBI's GenBank nucleotide database (Table S2 [suppl.]).

Statistical analysis

The frequency of fungal strains recovered from each site was calculated as a percentage and the frequency of different fungal taxa was numbered per host and per site. Frequency data (not normal distributions) obtained from oak species and different sites were subjected to chi-squared analysis using SAS software package (SAS Institute, Inc., USA, 2003). The species diversity among fungal communities was manually calculated using Excel software v. 2007 based on biodiversity indices including Shannon–Wiener index (H′), C and Margalef richness (D_{marg}).

Shannon–Wiener Index: $H' = -\sum_{i=1}^{S} P_i \log P_i \quad P_i = \frac{Ni}{N}$

Hill evenness: $E_H = \frac{H'}{N_1}$

Margalef richness: $D_{marg} = \frac{S-1}{\log N}$

Where N_i is number of individuals of each species in each community, N is the total number of individuals in community, S is the number of species encountered in each community. N_1 is Ln (N), *Pi* is the proportional abundance of the *i*th individual.

Results

A total of 94 fungal isolates comprising of 30 species were isolated from *Q. macranthera* and *Q. brantii* (Table 1). The majority of identified fungal species (29 species) belonged to the phylum Ascomycota, besides one basidiomyceteous isolate, *Phlebia radiata* (Table S2 [suppl.]). At least one representative of each taxonomic group (identified based on preliminary morphological features) was subjected to molecular identification based on ITS-rDNA or TUB sequence analysis. This allowed the placement of our sequenced isolates into ten orders (Pleosporales, Xylariales, Hypocreales, Sordariales, Diaporthales, Botryosphaeriales, Trichosphaeriales, Eurotiales, Pezizales and Polyporales), which belonged to 30 species (Fig. 1 and 2).

In phylogeny analysis, ITS-rDNA dataset (except *Fusarium* spp.) included 98 different in-group taxa and *Ganoderma tornatum* (CBS 109679) as the out-group taxon. The final single locus dataset comprised 972 characters (including alignment gaps), of which 635 characters were unique site patterns. MrModelTest v. 2.3 software recommended general time reversible (GTR) substitution as the best evolutionary model with gamma distribution, invariable sites and Dirichlet base frequencies. Bayesian inference of ITS-rDNA region resided our strains in 26 species, with the highest posterior probability (Fig. 1).

Beta-tubulin dataset for the phylogenetic analysis of *Fusarium* spp. consisted of 23 in-group taxa, *Penicillium araracuarense* (CBS 113149) as out-group taxon, and a total of 731 characters including 332 unique site patterns. MrModelTest v. 2.3 software selected Hasegawa-Kishino-Yano (HKY) substitution model as the best evolutionary model with gamma distribution and Dirichlet base frequencies. Based on the results, the identity of our strains was determined as *F. avenaceum*, *F. oxysporum*, *F. solani* and *F. proliferatum* (Fig. 2).

In this study, across the seven sampling counties, the numbers of 94 fungal isolates were recovered from both *Q. macranthera* (70 strains) and *Q. brantii* (24 strains) (Table 1 and Table S2). Chi-square analysis showed this frequency is significantly different between both hosts (Tables 2, 3). Proportional to the numbers of isolates, the most species diversity (24 taxa) was found among fungal community obtained from *Q. macranthera* (Table 4, Fig. 3) further corroborated by higher species diversity indices of Shannon–Wiener index (H′) and Margalef richness (D_{marg}) (Table 4). On the contrary, evenness (E_H) index for fungal community recovered from *Q. macranthera* was lower than *Q. brantii* (Table 4). It showed that the frequency of some taxa was higher among fungal community recovered from *Q. macranthera* (Tables 1 and 4, Fig. 3). Generally, these results highlight that barks of *Q. macranthera* is probably more preferable to be colonized by endophytic fungi than barks of *Q. brantii*.

Between provinces, 70 isolates were recovered from East Azerbaijan (67 isolates from *Q. macranthera* and 3 isolates from *Q. brantii*) and 24 isolates from Lorestan (3 isolates from *Q. macranthera* and 21 isolates from *Q. brantii*). This observation was further corroborated using Chi-squared analysis, so that a significant difference was detected between

Table 1. Frequency of occurrence (%) of the fungal endophytes obtained from surface-sterilized bark tissues of *Quercus macranthera* (Q1) and *Q. brantii* (Q2).

Isolated fungus	East Azerbaijan province				Lorestan province										Total (%)
	Kaleibar		Hatam-baig		Veisian		Shurab		Kaka Sharaf		Khorramabad		Chegani		
	Q1	Q2	Q1	Q2	Q1	Q2	Q1	Q2	Q1	Q2	Q1	Q2	Q1	Q2	
Alternaria alternata	6.38	0.0	7.44	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	13.82
Arthrinium arundinis	1.06	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.06
Biscogniauxia mediterranea	5.31	0.0	4.25	0.0	0.0	2.12	0.0	2.12	0.0	2.12	0.0	1.06	0.0	1.06	18.04
Epicoccum nigrum	1.06	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.06
Fusarium avenaceum	2.12	0.0	1.06	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.18
Fusarium oxysporum	1.06	0.0	1.06	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.12
Fusarium proliferatum	0.0	0.0	1.06	0.0	0.0	1.06	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.12
Fusarium solani	2.12	0.0	1.06	0.0	0.0	1.06	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.24
Nigrospora oryzae	3.19	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.19
Ochrocladosporium elatum	1.06	0.0	1.06	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.12
Pyronema domesticum	2.12	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.12
Sordaria fimicola	1.06	0.0	1.06	0.0	0.0	1.06	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.18
Sordaria sibutii	2.12	0.0	3.19	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.31
Valsa persoonii	0.0	0.0	0.0	0.0	0.0	1.06	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.06
Trichothecium roseum	4.25	0.0	5.31	0.0	1.06	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	10.62
Clonostachys rosea	1.06	0.0	0.0	0.0	0.0	1.06	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.12
Neoscytalidium dimidiatum	0.0	1.06	0.0	0.0	0.0	1.06	0.0	0.0	0.0	0.0	0.0	1.06	0.0	0.0	3.18
Daldinia vernicosa	1.06	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.06
Daldinia loculata	0.0	0.0	1.06	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.06
Daldinia palmensis	0.0	0.0	1.06	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.06
Chaetomium globosum	0.0	0.0	1.06	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.06
Discula quercina	0.0	0.0	1.06	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.06
Penicillium commune	0.0	0.0	1.06	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.06
Penicillium spinulosum	0.0	0.0	1.06	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.06
Paecilomyces variotii	0.0	0.0	0.0	1.06	0.0	1.06	0.0	1.06	0.0	0.0	0.0	0.0	0.0	0.0	3.18
Paecilomyces formosus	3.19	0.0	0.0	0.0	1.06	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.24
Phlebia radiata	0.0	0.0	0.0	0.0	0.0	1.06	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.06
Beauveria bassiana	0.0	0.0	0.0	0.0	0.0	1.06	0.0	1.06	0.0	0.0	0.0	0.0	0.0	0.0	2.12
Curvularia neergardii	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.06	1.06
Curvularia spicifera	0.0	0.0	0.0	1.06	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.06	2.12
Taxa diversity per host per county	16	1	16	2	2	10	0	3	0	1	0	2	0	3	30
Frequency of isolates per host per county	37	1	30	2	3	10	0	4	0	2	0	2	0	3	94
% frequency of isolates per host per county	39.3	1.06	31.91	2.13	2.13	10.64	0	4.26	0	2.13	0	2.13	0	3.19	100
Frequency of isolates per county	38		32		13		4		2		2		3		94
% frequency of isolates per county	40.42		34.04		13.82		4.25		2.12		2.12		3.19		100
% frequency of isolates per province	74.47								25.53						100

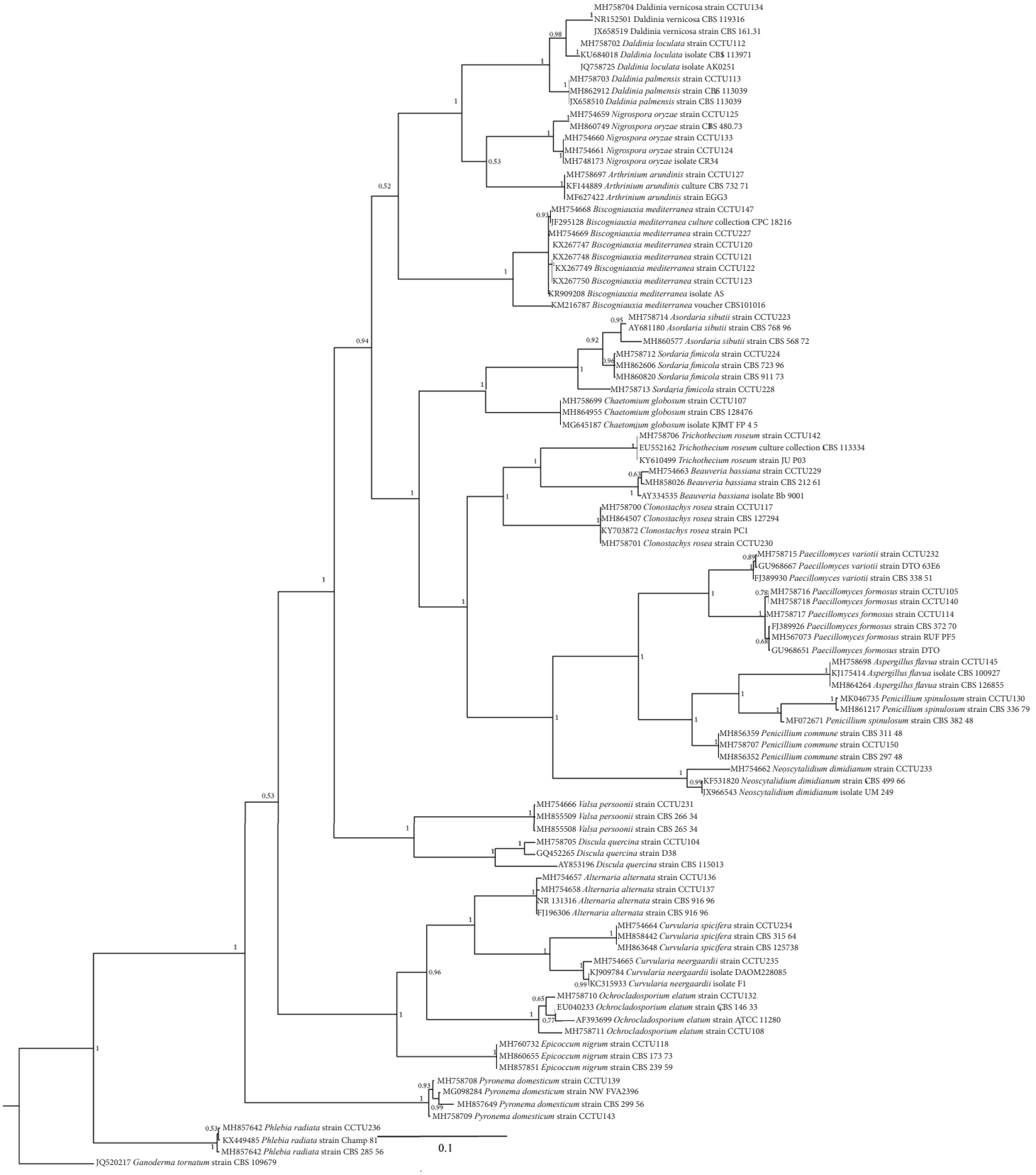

Figure 1. Bayesian inference phylogenetic tree of the ITS dataset belong to ascomycetous fungal taxa obtained in this study using MrBayes v. 3.2.1. The scale bar shows 0.09 expected changes per site. The tree was rooted to *Ganodermatornatum* (CBS 109697). Our isolates generated in this study are shown as CCTU.

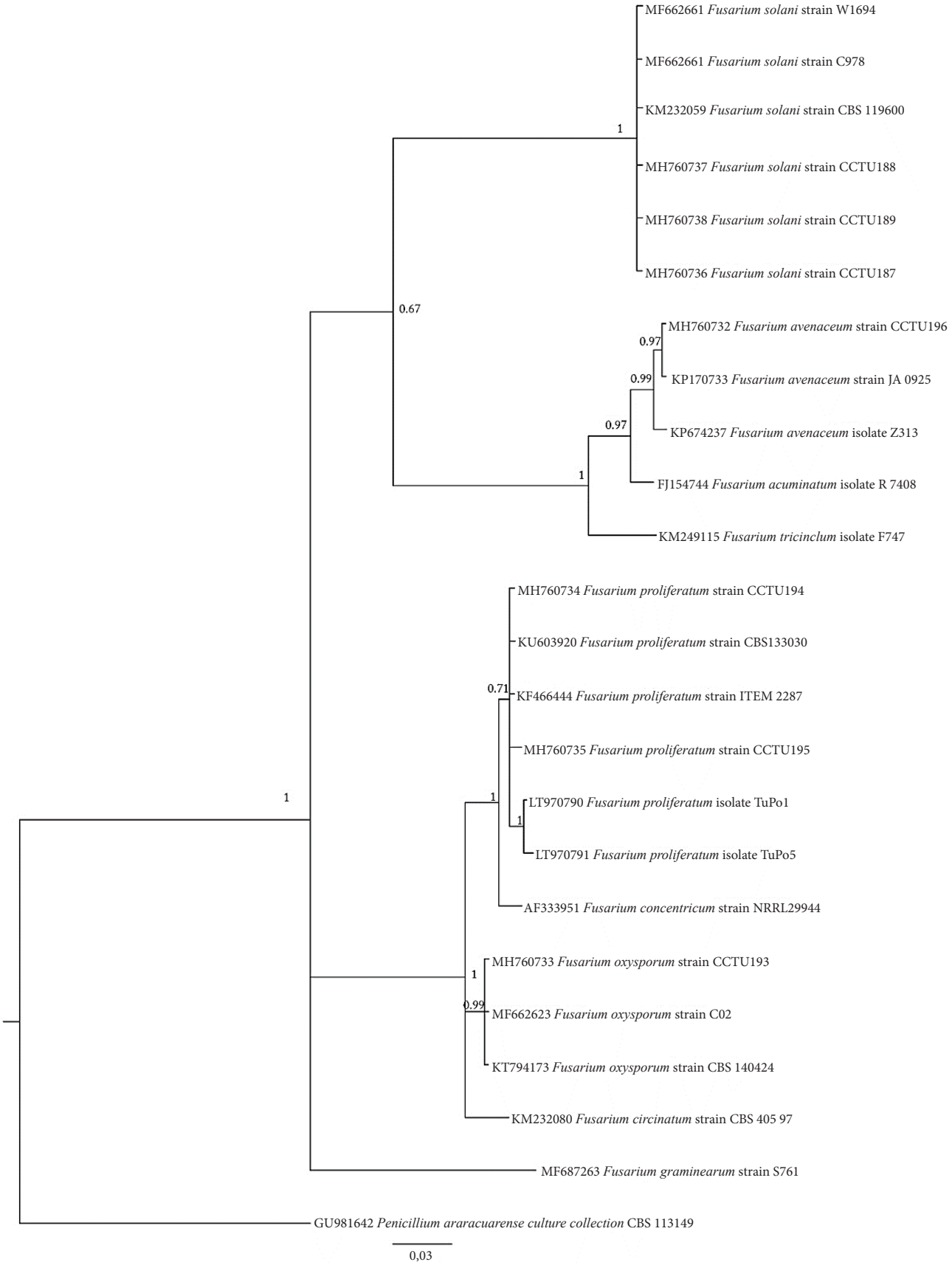

Figure 2. Bayesian inference phylogenetic tree of the β-tubulin dataset belong to *Fusarium* spp. obtained in this study using MrBayes v. 3.2.1. The scale bar shows 0.03 expected changes per site. The tree was rooted to *Penicilliumararacuarense* (CBS 113149). *Fusarium* spp. isolates generated in this study are shown as CCTU.

Table 2. Chi-squared values obtained from comparisons of frequencies of endophytic fungi recovered from *Quercus macranthera* and *Q. brantii* per location.

Locations	East Azarbaijan/ Lorestan provinces		Chi	df
	Q. macranthera	*Q. brantii*		
Kaleibar	37	1	34.1**	1
Hatam-baig	30	2	24.5**	1
Veisian	3	10	3.76	1
Shurab	0	4	4**	1
Kaka Sharaf	0	2	2**	1
Khorramabad	0	2	2**	1
Chegani	0	3	3**	1
Total	70	24	22.51**	1

** and * show significant different at level of 0.01 and 0.05 respectively.

Table 3. Chi-squared values obtained from comparisons of frequencies of endophytic fungi recovered from *Quercus macranthera* and *Q. brantii* between sampling locations. K: kaleibar; H: hatam-baig; V: veisian; S: shurab; Ks: kaka sharaf; Kh: khorramabad; Ch: chegani; ** and * show significant different at level of 0.01 and 0.05 respectively.

Host	East Azarbaijan	Lorestan								
	K-H	V-S	V-Ks	V-Kh	V-C	S-Ks	S-Kh	S-Ch	Ks-Kh	Ks-C	Kh-C
Q. macranthera	0.73	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Q. brantii	0.33	2.57	5.33**	5.33**	3.76	0.66	0.66	0.14	0.00	0.2	0.2
Total	0.51	4.76*	8.06**	8.06**	6.25*	0.66	0.66	0.14	0.00	0.2	0.2
Q. macranthera					38.72**						
Q. brantii					13.5**						
Total					22.51**						

Table 4. Values of diversity indices calculated on diversity of endophytic fungal taxa recovered from both species of *Quercus* spp. in different counties located in East Azerbaijan and Lorestan provinces.

Factors	No. of isolates	No. of taxa	Frequency (%)	Margalef richness (D_{marg})	Shannon–Wiener Index (H´)	Hill's evenness (E_H)
host						
Quercus macranthera	70	24	74.47	5.41	2.74	0.86
Quercus brantii	24	11	25.53	3.14	2.14	0.89
provinces						
East Azerbaijan	70	26	74.47	5.64	2.82	0.86
Lorestan	24	14	25.53	4.09	2.31	0.87
counties						
Kaleibar	38	17	40.42	4.39	2.47	0.87
Hatam-baig	32	18	34.04	4.9	2.35	0.81
Veisian	13	12	13.82	4.2	2.26	0.9
Shurab	4	2	4.26	1.44	1.01	0.91
Chegani	3	3	3.2	1.8	1.09	1
Kaka Sharaf	2	1	2.13	1.44	0	0
Khorramabad	2	2	2.13	1.44	0	0

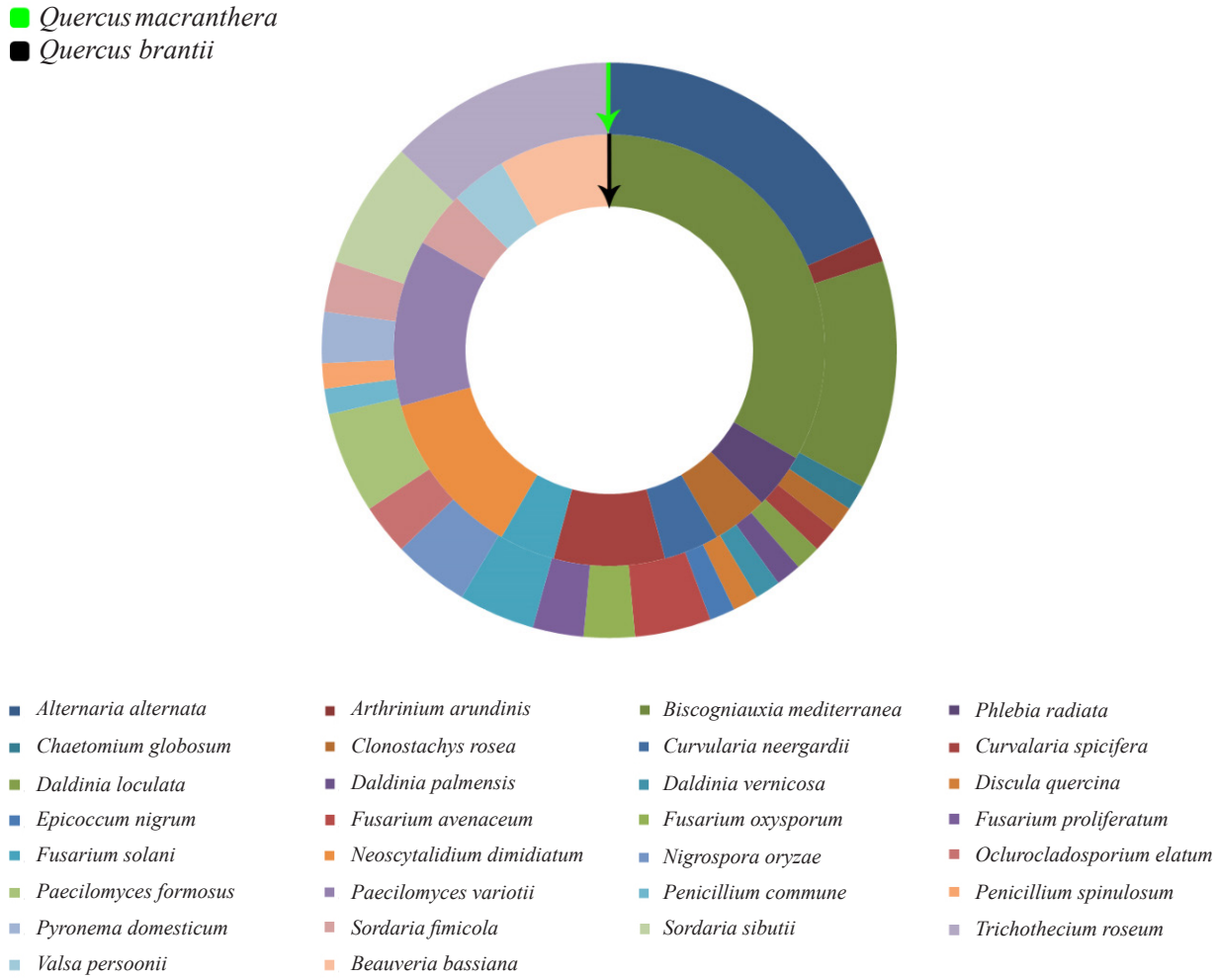

Figure 3. Frequency and diversity of fungal endophyte taxa recovered from both *Quercus macranthera* and *Q. brantii.*

provinces and even counties in terms of the numbers of fungal strains recovered from *Q. macranthera* and *Q. brantii* besides Veisian county (Table 3). In scale of counties, the highest frequency of isolates was found in Kaleibar and Hatam-baig counties in East Azerbaijan and followed by Veisian in Lorestan province (Table 4). Moreover, the highest species diversity was also detected in fungal community of Hatam-baig and Kaleibar in East Azerbaijan and followed by Veisian further confirmed by biodiversity indices (Table 4). This highlights the significant effect of growth location on the frequency and diversity of fungal endophyte community of *Quercus* in Iran.

Discussion

Overall, the observations of this study suggest that both plant species and plant growth location are involved in distribution and diversity of fungal endophytic communities of *Quercus* in Iran. It appears that higher frequency and diversity of fungal endophytic community on *Q. macranthera* in East Azerbaijan is probably due to either old establishment of *Q. macranthera* in East Azerbaijan or more favorable atmospheric condition of East Azerbaijan (mountainous and temperate climate) for establishment of this plant species and fungal communities. In the present study all samplings and isolations were made during summer 2014, thus, differences between isolation frequencies cannot be due to date of sampling. Giauque and Hawkes (2013) have examined the relative importance of environmental and spatial factors in structuring endophyte communities of *Panicum hallii* Vasey and *P. virgatum* L. They concluded that environmental factors related to historical and current precipitation were the most important predictors of endophyte communities. In a survey of endophytic fungal communities in leaves of *Metrosideros polymorpha* Gaudich. across wide environmental gradients in

Hawaiian landscape, among-site variation in endophyte community composition was found to be correlated strongly with temperature and rainfall (Zimmerman & Vitousek, 2012).

The most frequent fungal species recovered from across the counties were *Biscogniauxia mediterranea*, *Alternaria alternata*, *Trichothecium roseum*, *Sordaria sibutii* and *Paecilomyces formosus*. *Biscogniauxia mediterranea* had the highest relative frequency (18.28%) recovered from *Q. macranthera* and *Q. brantii* in all counties (Table 1). This fungus has been shown to be a latent pathogen, with potential to cause major losses to oak industry in Iran (Mirabolfathy, 2013). *Alternaria alternata* which is frequently identified as endophyte (Ragazzi *et al*., 2001; Selim *et al*., 2011; Maheswari & Rajagopal, 2013; Nalini *et al*., 2014) was the second most frequent endophyte and followed by *Trichothecium roseum*. Different endophytic fungal taxa showed different relative frequencies in two oak species (or different locations). *Quercus macranthera* yielded the greater fungal diversity, with 24 different taxa being isolated (Tables 1, 4 and Table S2). Some of the endophytic species were found in only one host species, some are cosmopolitan, not specific to oak and some are rarely found. It shows that these fungal taxa could either restrict only to those counties or may have spread recently across those counties. For example, the only isolate of *Curvularia neergardii* came from *Q. brantii*. Furthermore, three species of *Daldinia* with a relative frequency of 4.28% were only obtained from *Q. macranthera*. The composition and abundance of the endophytes varied according to the host tested. Although the data may indicate that, some of fungal endophytes dominate in mycobiota of *Quercus* spp., whether it is a result of natural selection or not, awaits detailed investigations.

To the best of our knowledge all of the species identified in this study, except *B. mediterranea* (Davari *et al*., 2003; Mirabolfathy, 2013), are reported for the first time from *Q. macranthera* and *Q. brantii*. Recently, Hajizadeh *et al*., (2015) have studied species diversity of fungal endophytes of *Q. brantii* in Kurdistan province, Iran. They reported *Cladosporium tenellum*, *Paecilomyces formosus*, *Petriella guttulata*, *Preussia australis*, and *Sordaria sibutii*. This is the first report of *Pyronema domesticum* and *Valsa persoonii* for the mycobiota of Iran. To the best of our knowledge, this is the first survey of cultivable endophytic fungal community of *Q. macranthera*. Several investigations have been conducted regarding fungal endophytes of different oak species (Ragazzi *et al*., 2001; Anselmi *et al*., 2004; Kwasna *et al*., 2016). Kwasna *et al*. (2016) characterized root fungal endophytes of *Q. rubor*. They identified a more diverse fungal species including 126 taxa (Zygomycota, Ascomycota and Basidiomycota), and number of species was higher in roots subjected to floods. It seems that the studied tissue (root) had an effect on species diversity of isolated endophytes. In 2001, endophytes of current-year twigs, buds and leaves of *Q. cerris* were investigated and the results revealed organ specificity for endophytic fungi (Ragazzi *et al*., 2001).

In the assemblage of endophytic fungi in healthy tissues of oak trees, some of them may be possible latent pathogens of oak. Our data revealed a low proportion of strains of oak phytopathogenic fungi. However, *B. mediterranea* and *Ph. radiata*, usually associated with oak decline were isolated (Boddy & Rayner, 1983; Mirabolfathy, 2013). *Biscogniauxia mediterranea* is mainly related to charcoal disease (Mirabolfathy, 2013). Interestingly, no wood-decaying basidiomycetes associated with oak trees were recovered in East Azerbaijan province. Some of the recovered genera in this study have previously been reported as potential biocontrol agents, which draws attention to further clarification of their antimicrobial properties (Gonzalez & Tello, 2011). Of those, several species belonging to genera such as *Chaetomium* (*Ch. globosum*), *Epicoccum* (*E. nigrum*) and *Fusarium* (*F. proliferatum*) have been here obtained. *Neoscytalidium dimidiatum* was only isolated from *Quercus brantii* in this study. Bakhshizadeh *et al*., (2014) have reported *N. dimidiatum* as a human pathogen from Iran. This highlights that further investigations are needed to fully elucidate the ecology and putative use of wood-inhabiting endophytes.

Although endophytic fungi are known to be ubiquitously distributed in terrestrial plants and the plant itself benefits from these hidden inhabitants as they modulate host nutrition, metabolites, and stress response (Yuan *et al*., 2010; Soltani *et al*., 2016), only recently, intense research efforts have been sought to build a more detailed understanding of biodiversity and bioprospecting of endophytic fungi (Aly *et al*., 2010; Soltani *et al*., 2016). Herein, we focused on cultivable fungal species, however uncultivable strains could be a big portion of endophytic fungal community. Since those strains could be, for example the candidate fungi for production bioactive molecules (Tejesvi *et al*., 2011), future surveys should focus on metagenomics and transcriptomics approaches to study the functional role of those hidden members of the microbial population.

Conclusions

The frequency and diversity of fungal community recovered from *Q. macranthera* and East Azerbaijan

province was far higher than *Q. brantii* and Lorestan province respectively. Accordingly, our data and analyses demonstrate that both oak species and growth locations play a prominent role in shaping the frequency and diversity of fungal endophyte community of *Quercus* in Iran.

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References

Aly AH, Debbab A, Kjer J, Proksch P, 2010. Fungal endophytes from higher plants: a prolific source of phytochemicals and other bioactive natural products. Fungal Divers 41: 1-16. https://doi.org/10.1007/s13225-010-0034-4

Anselmi N, Cellerino GP, Franceschini A, Granata G, Luisi N, Marras F, Mazzaglia A, Mutto Accordi S, Ragazzi A, 2004. Geographic distribution of fungalendophytes of Quercus sp. in Italy. In: Endophytism in Forest Trees; Ragazzi A, Moricca S, Dellavalle I (eds). pp: 73-89. AISF, Florence, Italy.

Arnold AE, Lutzoni F, 2007. Diversity and host range of foliar fungal endophytes: are tropical leaves biodiversity hotspots? Ecol 88: 541-549. https://doi.org/10.1890/05-1459

Arnold AE, Engelbrecht BMJ, 2007. Fungal endophytes nearly double minimum leaf conductance in seedlings of a neotropical tree species. J Trop Ecolo 23: 369-372. https://doi.org/10.1017/S0266467407004038

Arnold AE, Mejia LC, Kyllo D, Rojas EI, Maynard Z, Robbins N, Herre EA, 2003. Fungal endophytes limit pathogen damage in a tropical tree. Proc Natl Acad Sci U.S.A: PANS. pp: 100, 15649-15654.

Arnold AE, 2001. Fungal endophytes in neotropical trees: abundance, diversity, and ecological interactions. In: Tropical ecosystems: structure, diversity, and human welfare; Ganeshaiah KN, Uma Shaanker R, Bawa KS (eds). pp: 739-743. Oxford and IBH Publishing Co., New Delhi, India.

Arnold AE, Maynard Z, Gilbert GS, Coley PD, Kursar TA, 2000. Are tropical fungal endophytes hyperdiverse? Ecol 3: 267-274. https://doi.org/10.1046/j.1461-0248.2000.00159.x

Arzanlou M, Khodaei S, 2012. Aureobasidium iranianum, a new species on bamboo from Iran. Mycosphere 3: 404-408. https://doi.org/10.5943/mycosphere/3/4/2

Bakhshizadeh M, Hashemian HR, Najafzadeh MJ, Dolatabadi S, Zarrinfar H, 2014. First report of rhinosinusitis caused by Neoscytalidium dimidiatum in Iran. J Med Microbiol 63: 1017-1019. https://doi.org/10.1099/jmm.0.065292-0

Bayman P, Angulo-Sandoval P, Baez-Ortiz Z, Lodge DJ, 1998. Distribution and dispersal of Xylaria endophytes in two tree species in Puerto Rico. Mycol Res 102: 143-149 https://doi.org/10.1017/S095375629700590X

Blumenstein K, 2010. Characterization of endophytic fungi in the genus Ulmus: putativeagents for the biocontrol of Dutch elm disease (DED). Diploma thesis, University of Kassel, Kassel, Germany.

Boddy L, Rayner ADM, 1983. Mycelial interactions, morphogenesis and ecology of Phlebia radiata and P. rufa from oak. Trans Br Mycol Soc 80 (3): 437-448. https://doi.org/10.1016/S0007-1536(83)80040-0

Bills GF, Polishook JD, 1992. Recovery of endophytic fungi from Chamaecyparis thyoides. Sydowia 44: 1-12.

Cannon CD, Simmons CM, 2002. Diversity and host preference of leaf endophytic fungi in the Iwokrama forest reserve, Guyana. Mycologia 94: 210-220. https://doi.org/10.1080/15572536.2003.11833226

Carroll GC, Carroll FE, 1978. Studies on the incidence of coniferous needle endophytes in the Pacific Northwest. Can J Bot 56: 3034-3043. https://doi.org/10.1139/b78-367

Costa Pinto LS, Azeved JL, Pereira JO, Carneiro Vieira ML, Labate CA, 2000. Symptomless infection of banana and maize by endophytic fungi impairs photosynthetic efficiency. New Phyto 147: 609-615. https://doi.org/10.1046/j.1469-8137.2000.00722.x

Davari M, Payghami E, Javanshir A, Ebrahimi T, 2003. Etiology of oak (Quercus macranthera) decline in Hatam-Baig forest of Meshkinshahr area. Agric Sci (Tabriz) 13(3): 1-14.

Estrada C, Wcislo WT, Van Bael SA, 2013, Symbiotic fungi alter plant chemistry that discourages leaf-cutting ants. New Phytol 198: 241-251. https://doi.org/10.1111/nph.12140

Fisher PJ, Petrini O, Petrini LE, Sutton BC, 1994. Fungal endophytes from the leaves and twigs of Quercus ilex L. from England, Majorca and Switzerland. New Phytol 127: 133-137. https://doi.org/10.1111/j.1469-8137.1994.tb04267.x

Fisher PJ, Graf F, Petrini LE, Sutton BC, Wookey PA, 1995. Fungal endophytes of Dryas octopetala from a high polar semidesert and from the Swiss Alps. Mycologia 87: 319-323. https://doi.org/10.1080/00275514.1995.12026536

Gamboa MA, Bayman P, 2001. Communities of endophytic fungi in leaves of a tropical timber tree (Guarea guidonia: Meliaceae). Biotropica 33:352-360. https://doi.org/10.1111/j.1744-7429.2001.tb00187.x

Frohlich J, Hyde KD, 1999. Biodiversity of palm fungi in the tropics: are global fungal diversity estimates realistic? Biodivers Conserv 8: 977-1004. https://doi.org/10.1023/A:1008895913857

Giauque H, Hawkes CV, 2013. Climate affects symbiotic fungal endophyte diversity and performance. Amer J Bot 100 (7): 1435-44. https://doi.org/10.3732/ajb.1200568

Glass NL, Donaldson GC, 1995. Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. Appl Environ Microbiol 61:1323-1330.

Gonzalez V, Tello ML, 2011. The endophytic mycota associated with Vitis vinifera in central Spain. Fungal Divers 47: 29-42. https://doi.org/10.1007/s13225-010-0073-x

Hajizadeh A, Amini J, Abdollahzadeh J, 2015. New records of endophytic fungi isolated from oak trees in Kurdistan province (Iran). Rostaniha 16(1): 109-122.

Hata K and Futai K, 1996. Variation in fungal endophyte populations in needles of the genus Pinus. Can J Bot 74: 103-114. https://doi.org/10.1139/b96-015

Helander M, Ahlholm J, Sieber TN, Hinneri S, Saikkonen K, 2007. Fragmented environment affects birch leaf endophytes. New Phytol 175: 547-553. https://doi.org/10.1111/j.1469-8137.2007.02110.x

Higgins KL, Arnold AE, Miadlikowska J, Sarvate SD, Lutzoni F, 2007. Phylogenetic relationships, host affinity, and geographic structure of boreal and arctic endophytes from three major plant lineages. Mol Phylogenet Evol 42: 543-555. https://doi.org/10.1016/j.ympev.2006.07.012

Karimi K, Khodaei S, Rota-Stabelli O, Arzanlou M. Pertot I, 2016. Identification and Characterization of two new Fungal Pathogens of Polygonatum odoratum (Angular Solomon's seal) in Italy. J Phytopathol 164: 1075-1084. https://doi.org/10.1111/jph.12528

Katoh K, Toh H, 2008. Recent developments in the MAFFT multiple sequence alignment program. Brief Bioinform 9: 286-298. https://doi.org/10.1093/bib/bbn013

Kwasna H., Szewczyk W., Behnke-Borowczyk J, 2016. Fungal root endophytes of Quercus robur subjected to flooding. For Pathol 46: 35-46.

Maheswari S, Rajagopal K, 2013. Biodiversity of endophytic fungi in Kigelia pinnata during two different seasons. Curr Sci 104 (4): 515-518.

Mejia LD, Rojas EI, Maynard Z, Arnold AE, Van Bael SA, Samuels GJ, Robbins N, Herre EA, 2008. Endophytic fungi as biocontrol agents of Theobroma cacao pathogens. Biol Control 46: 4-14. https://doi.org/10.1016/j.biocontrol.2008.01.012

Mirabolfathy M, 2013. Outbreak of charcoal disease on Quercus spp. and Zelkova carpinifolia trees in forests of Zagros and Alborz mountains in Iran. Iran J Plant Pathol 49 (2): 77-79.

Mohali S, Burgess TI, Wingfield MJ, 2005. Diversity and host association of the tropical tree endophyte Lasiodiplodia theobromae revealed using simple sequence repeat markers. For Pathol 35: 385-396.

Möller EM, Bahnweg G, Sandermann H, Geiger HH, 1992. A simple and efficient protocol for isolation of high molecular weight DNA from filamentous fungi, fruit bodies, and infected plant tissues. Nucleic Acids Res 20: 6115-6116. https://doi.org/10.1093/nar/20.22.6115

Nalini MS, Sunayana N, Prakash HS, 2014. Endophytic Fungal Diversity in Medicinal Plants of Western Ghats, India. Int J Biodivers 2014, 1-9. https://doi.org/10.1155/2014/494213

Nylander JAA, 2004. MrModeltest v. 2.0. Program distributed by the author. Evolutionary Biology Centre, Uppsala University, Uppsala, Sweden.

Petrini O, Stone J, Carroll FE, 1982. Endophytic fungi in evergreen shrubs in western Oregon: a preliminary study. Can J Bot 60: 789-796. https://doi.org/10.1139/b82-102

Petrini O, Sieber TN, Toti L, Viret O, 1992. Ecology, Metabolite Production, and Substrate Utilization in Endophytic Fungi. Nat Toxins 1: 185-196. https://doi.org/10.1002/nt.2620010306

Petrini O, 1996. Ecological and physiological aspects of host-specificity in endophytic fungi. In: Endophytic Fungi in Grasses and Woody Plants: Systematics, Ecology and Evolution; Redlin SC, Carris LMSt, Paul MN (eds). pp: 87-100. APS Press, New York, USA.

Ragazzi A, Moricca S, Capretti P, Dellavalle I, Mancini F, Turco E, 2001. Endophytic fungi in Quercus cerris: isolation frequency in relation to phenological phase, tree health and the organ affected. Phytopathol Mediterr 40: 165-171.

Rambaut A, 2009. FigTree v1.3.1. Internet Resource: http://tree.bio.ed.ac.uk/software/figtree/

Ronquist F, Huelsenbeck JP, 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1572-1574. https://doi.org/10.1093/bioinformatics/btg180

Saikkonen K, Faeth SH, Helander M, Sullivan TJ, 1998. Fungal endophytes: a continuum of interactions with host plants. Annu Rev Ecol Evol Syst 29: 319-343. https://doi.org/10.1146/annurev.ecolsys.29.1.319

Schardl CL, Leuchtmann A, Spiering MJ, 2004. Symbioses of grasses with seedborne fungal endophytes. Annu Rev Plant Biol 55: 315-340. https://doi.org/10.1146/annurev.arplant.55.031903.141735

Seifert K, Morgan-Jones G, Gams W, Kendrick B, 2011. The genera of hyphomycetes. CBS Biodiversity Series no. 9. CBS-KNAW Fungal Biodiversity Centre, Utrecht. 997 pp.

Selim KA, El-Beih AA, Abd-El-Rahman TM, El-Diwany AI, 2011. Biodiversity and antimicrobial activity of endophytes associated with Egyptian medicinal plants. Mycosphere 2 (6): 669-678. https://doi.org/10.5943/mycosphere/2/6/7

Sieber TN, 1989. Endophytic fungi in twigs of healthy and diseased Norway spruce and white fir. Mycol Res 92: 322-326. https://doi.org/10.1016/S0953-7562(89)80073-5

Soltani J, Zaheri-Shoja M, Hamzei J, Hosseyni-Moghaddam MS, Pakvaz S. 2016. Diversity and bioactivity of bacterial endophyte community of Cupressaceae. For Pathol 46 (4): 353-361. https://doi.org/10.1111/efp.12270

Suryanarayanan TS, Kumaresan V, 2000. Endophytic fungi of some halophytes from an estuarine mangrove forest. Mycol Res 104: 1465-1467. https://doi.org/10.1017/S0953756200002859

Suryanarayanan TS, Wittlinger SK, Faeth SH, 2005. Endophytic fungi associated with cacti in Arizona. Mycol Res 109: 635-639. https://doi.org/10.1017/S0953756205002753

Sutton BC, 1980. The Coelomycetes. Fungi Imperfecti with Pycnidia, Acervuli and Stromata. CMI, Kew. 696 pp.

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S, 2013. MEGA 6: Molecular Evolutionary Genetics Analysis Version 6.0. Mol Biol Evol 30: 2725-2729. https://doi.org/10.1093/molbev/mst197

Tejesvi MV, Kajula M, Mattila S, Pirttila AM, 2011. Bioactivity and genetic diversity of endophytic fungi in Rhododendron tomentosum Harmaja. Fungal Divers 47: 97-107. https://doi.org/10.1007/s13225-010-0087-4

White TJ, Bruns T, Lee S, Taylor J, 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR protocols: a guide to methods and applications; Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds). pp: 315-322. APS press, New York, USA: AP. https://doi.org/10.1016/B978-0-12-372180-8.50042-1

Yuan ZL, Zhang CL, Lin FC, Kubicek CP, 2010. Identity, diversity, and molecular phylogeny of the endophytic mycobiota in the roots of rare wild rice (Oryza granulate) from a nature reserve in Yunnan, China. Appl Environ Microbiol 76 (5): 1642-1652. https://doi.org/10.1128/AEM.01911-09

Zimmerman NB, Vitousek PM, 2012. Fungal endophyte communities reflect environmental structuring across a Hawaiian landscape. Proc Nation Acad Sci U.S.A: PNAS. pp: 109 (32): 13022-13027.