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MYCORRHIZAL FUNGI OF SOME *ORCHIS* SPECIES OF TURKEY

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Abstract

In Turkey, orchids are under a serious threat of extinction as a result of both excessive collecting and severe destruction of their habitats. Studies conducted have shown that the protection of orchids depends on the presence of mycorrhizal fungal biodiversity. The purpose of this study is to isolate the orchid mycorrhizal fungi in the roots of the individuals of 7 *Orchis* species taken from three different habitats in Samsun (Turkey) and to create a fungus bank after molecular identification is conducted. For this reason, the plant roots of *Orchis* species were collected at the spring and summer months of 2014 and 2015. Isolates were classified according to morphological characteristics and examined according to rDNA ITS sequences. Nine isolates were defined morphologically as *Rhizoctonia*-like, while one isolate was defined as non- *Rhizoctonia*. Cluster analysis (UPGMA) method was used to find out the similarities and differences of morphological characteristics and cladogram was constructed. In order to be able to define the isolates of 3 clads formed with morphological data at molecular level, we obtained ITS1-5.8S-ITS4 area by using ITS 4 and ITS 5 primers. Based on DNA sequencing, it was found that 7 of the 9 *Rhizoctonia*-like isolates were associated with sequences of *Tulasnellaceae* species while 2 were associated with sequences of *Ceratobasidiaceae* species and 1 non-*Rhizoctonia* isolate was associated with sequences of *Pezizaceae* species.

Key words: *Orchis,* Orchid mycorrhiza, Symbiosis, *Tulasnella*.

Introduction

Orchidaceae is one of the widespread plant families and it can be found all over the world, except for poles and some deserts (Cribb *et al.,* 2003). However, many orchid species are described by restricted geographic distributions (Tremblay *et al.,* 2004; Molnar *et al*., 2017). Orchids propagate via seed germination in natural conditions. All orchids investigated to date demonstrated that obligate relationship with mycorrhizal fungi is necessary for the seed germination (Leake, 1994; Rasmussen, 1995). A great majority of fungi are included within Basidiomycota (Rasmussen, 1995, 2002; Taylor *et al.,* 2002) and Ascomycota (Taylor &Bruns, 1997, 1999; Taylor *et al.,* 2002). The degree of the specificity between orchid and fungus is the predominant agent determining the seedling establishment chance (Bidartondo& Read, 2008). In other words, mycorrhizal fungi are an indispensable part of the orchid life cycle; however, little is known about mycorrhizal fungal diversity (Jacquemyn *et al*., 2015; Esposito *et al.,* 2016). Consequently, it can be suggested that the dispersion and abundance of orchids in their habitats is related to the distribution and the abundance of their mycorrhizal fungi (Jacquemyn *et al.,* 2012; McCormick &Jacquemyn, 2014; Esposito *et al.,* 2016; Jacquemyn *et al.,* 2016). A characteristic of the populations of many orchid species is the small number of individuals in the habitat and probably, the rarity of the orchid populations and distribution of specific, required fungi are interrelated (Waterman & Bidartondo 2008; McCormick *et al.,* 2016). Two applications are used to detect the dispersion of orchid fungi; first, isolation of fungi from the roots of orchids and second, burial of seed packages in soil (Rasmussen & Whigham, 1993). Isolated fungi have been identified via morphological and molecular techniques (Masuhara & Katsuya, 1994; Zettler *et al.,* 2011). However, correlation between OMF and distribution of orchids is still not fully defined.

In addition to this, more than one mycorrhizal fungus can be found in the roots of the same orchid individual permanently or in seasonal periods (Girlanda *et al.,* 2006). Especially in Turkey, there are about 200 orchid species. All orchids in Turkey (endemic or not) are under protection legally and although it is prohibited to collect the bulbs, they are collected excessively each year for salep and ice-cream production (Sezik, 2002). On the other hand, there are not enough studies about the seed germination physiology of orchids and mycorrhizal fungal variety for the purposes of both production and protection. For orchids to be produced and to be taken under protection, the mycorrhizal fungi of these species should be determined first and an orchid fungus bank should be established. With our previous study which was conducted in this aspect, mycorrhizal fungi in some species had been determined (Kompe & Mutlu, 2017). With the current study, we are continuing our studies in this aspect. Our purpose in this study is to make contributions to finding out the association between mycorrhizal fungal variety of *Orchis* species and their habitats and to obtain fungal material for future studies of production in the soil from seed. The fungal materials obtained will also be included in the orchid fungus bank.

Material and Method

Sampling was conducted to be able to find out the mycorrhizal fungus of the orchids spread in three different habitats in Samsun as wet meadow (*Orchislaxiflora, Orchispalustris*), dune area (*Orchiscoriophora*) and *Quercus* forest side (*Orchispapillionacea, Orchispurpurea, Orchissimia, Orchistridentata*). The areas from which root samples were taken are shown in Fig. 1. Identifications of *Orchis* species were made according to Flora of Turkey (Davis, 1984). Healthy roots of the plants were collected during the flowering period, in the spring and early summer of 2014 and 2015. From single population of each orchid species, five individual plants were randomly selected and two root samples of each orchid plant were collected.

Fig. 1. Sampling areas in Samsun.

The infected root parts were surface sterilized with 1,5% NaOCl for 2-3 minutes and then washed with sterilized distilled water three times, thin cross-sections taken from roots were placed on modified fungus isolation medium in the petri dishes in laminar flow and incubated at 26±2ºC (Clements *et al.*, 1986). The growing fungal hyphae were isolated from the roots according to the method described in Sneh *et al.,* (1991). The fungal hyphae stained with lactophenol cotton blue (LPCB) and hyphae were assessed microscopically using light microscopy (Lecia 134 microscope). The number of nuclei per cell was detected according to the method described in Bandoni's (1979). The color of colony and sclerotia was determined according the color chart of the Royal Horticultural Society of London.

Genomic DNA was extracted from fungal isolates by using the technique described by Pascual *et al.,* (2000). DNA was extracted from 50 mg of the fungal tissue. For pure cultures of mycorrhizal fungi, the ITS1 and ITS2 regions of fungal rDNA was amplified by PCR with the primers ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) and ITS5 (5′-GGAAGTAAAAGTCGAACAAGG-3′) were used for amplification according to White *et al.,* (1990).

The amplicons from the fungal isolates were sequenced by Macrogen (Korea) using an ABI 3730 XL sequencer. The primers ITS1 and ITS4 were used as sequencing primers and the amplicons were sequenced on both strands. The sequences were aligned with Clustal X. (Thompson *et al.,* 1997) and optimized manually. Firstly, the identitises of isolates were determined by making BLAST search. The sequences of the ITS-5.8S amplicons were blasted against the NCBI nucleotide database to detect published sequences with a high similarity.

Data analysis

Morphological characterizations (quantitative and qualitative) were appraised as described by Pereira *et al*., 2009. Quantitative and qualitative characteristics were examined by biometric techniques to cluster the isolates using the PAST software (Hammer *et al.,* 2001).

Morphological characteristics were analyzed by Unweighted Pair Group Method using Arithmetic Averages (UPGMA) method using cluster analysis (Cruz, 2008).

Phylogeny analysis of sequence results were constructed using the package program jModeltest v.0.1 (Guindon & Gascuel, 2003; Posada, 2008), Mega 6 software (Tamura *et al.,* 2013) and the package program Mr. Bayes version 3.2 (Ronquist *et al.,* 2012).

Results

Fungal isolates were obtained as a result of isolations conducted from the healthy three roots of five plants of each orchid species. These isolates were grouped according to their morphological features. The isolates were grouped in two as *Rhizoctonia*-like and non-*Rhizoctonia* (Fig. 2a, b, Table 1). Morphological features of the isolated fungi in both groups (hyphae diameter, number of nuclei, colony color, colony appearance) were determined. Three different colony colors of isolates which were left to incubation at 26±2°C in the dark for two weeks in a PDA medium were determined. While the most frequently observed colony color was grayish yellow, other less observed colony colors were grayish orange and yellowish white. Colony appearance was described as frequently velvety and rarely wooly. Hypha diameter varied between 2.830 and 4.489. Colony color of the non-*Rhizoctonia* isolates were determined as grayish-yellow, while the colony appearance was determined as wooly. Vegetative hypha color of all isolates was transparent (Table 1). *Rhizoctonia*-like fungi were found to be binucleate (BN), while non *Rhizoctonia* fungi were found to be multinucleate (MN) (Table 1). The number of groups increased to 3 within the interval of 60-75%. The first group included MN non-*Rhizoctonia* (OL2) isolate with radial zoning and grayish orange coloring, wooly appearance and aerial hypha on the border of petri; the second group included BN *Rhizoctonia*-like (OP1, OC2, OC3, OPL1, OPr1, OT1, OL1) isolates with grayish yellow colony color, velvety-wooly changing appearance and non-aerial hypha and the third group included other BN *Rhizoctonia*-like (OC1 and OS1) isolates with yellowish white color, velvety appearance and non-aerial hypha (Fig. 2a).

Cluster analysis (UPGMA) method was used to find out the similarities and differences of morphological characteristics between taxa and samples taken from different localities of the same taxon and cladogram was constructed. According to the results of the clustering analysis, taxa were grouped in two groups which were very far from each other within 0-12% similarity interval (Fig. 3).

For the molecular identification of the isolates of 3 clads constructed with morphological data, ITS4-ITS5 primary pair PCR product was formed. As a result of running isolates, the ITS1-5.8S-ITS2' rDNA gene areas of which were reproduced, on electrophoresis, products reproduced between 410-644 bp were obtained. Serial analysis of ITS1-5.8S-ITS2 gene area products of *isolates* reproduced with ITS4 and ITS5 primers was conducted by Macrogencompany. Consensus sequences were obtained by using Sequencher 4.7 Demo program. Consensus sequences of the isolated were compared with sequences stored in the Genebank (NCBI) by using Blast analysis. According to BLAST results, 7 of the 9 *Rhizoctonia*-like isolates were found to show 99% resemblance to *Tulasnellaceae* species, while 2 were found to show 99% resemblance to *Ceratobasidiaceae* species and 1 non-*Rhizoctonia* isolate was found to show 99% resemblance to *Pezizaceae* species. Closely related series which showed high similarity values between sequences (99%) were

added in the files formed with the consensus sequences of our isolates (Table 2). Modeltest v.0.1 program was used for the correct selection of base change model in the prediction of evolutionary parameters and the formation of phylogenetic trees. When analyzed with ModelTest v.0.1 program, it was found with hierarchical probability tests that the most suitable base change model for ITS *Tulasnella*, *Ceratobasidiaceae* and *Pezizaceae* isolates was Kimura-2 and the gamma shape values were found to be 0.419, 0.328 and 0.58, respectively. With the determined models, the trees formed with the evolutionary model which gave the highest Bootstrap values in Neighbor-Joining (NJ) and Maksimum-Likelihood (ML) analyses by using document opened in fasta format in Mega 6 program and the trees formed in Bayesian Information Criterion (BIC) analyses were formed. Phylogenetic trees formed were used by combining in NJ tree (Figs. 4, 5, 6).

Based on the phylogenetic tree, isolates of the *Tulasnella* species were grouped in two main clads. The data set formed for *Tulasnella* species included the sequence of 7 fungal isolates and the 21 reference sequences taken from NCBI. In this study, NJ and ML trees include 28 nucleotide sequence. There are a total of 128 positions in the data set. *Tulasnella* tree was grouped in three clads. OL1 and OC3 sequences (75% NJ/ 78% ML/ 0.78% Mr Bayes) are associated with *Tulasnella bifrons* (AY373290.1) OL1 and OC3 sequences are associated with *T. bifrons* (AY373290.1) (Fig. 4). OP 1 and OT1 sequences are found to be closely associated with the clad *T. violea* (KC152434), *T. tomaculum* (KC152380) and *T. eichlerina* (AY373292) species are clustered in (99% NJ /99% ML /0.90% MRbayes) (Fig. 4). OC2 and OPr1 sequences are found to show high similarity to uncultured *Tulasnella* (KC243938) isolated from *Gymnadenia conopsea* (92% NJ /94% ML /0.95% Mr Bayes) (Fig. 4).

The isolates of *Ceratobasidium* species were grouped in two main clads with a bootstrap value of 59% NJ/ 85% ML /0.70% Mr Bayes, respectively. The data set formed for *Ceratobasidium* members have the sequence of 2 fungal isolates and 33 reference sequences taken from NCBI.The sequence of OS1 isolate is associated with the clad *C. obscurum* (EU288894) clusters in (75% NJ /78% ML /0.78%MRbayes). OC 1 sequence is associated with the clad *C. albasitensis* (HQ680963) clusters in (83%NJ/ 84% ML /0.90% Mr Bayes) (Fig. 5).

OL2 sequence has 99% similarity rate with *Pezizaceae* species stored in GenBank with the access number of AJ242881. For the phylogenetic tree of *Pezizaceae* species, 26 sequences associated with *Pezizaceae* family were formed. It was grouped in two main clads supported with 100% Bootsprap value. OL2 sequence was placed in *Pezizaceae* clad. In the phylogenetic tree, OL2 isolate isolated from *Anacamptis laxiflora* roots was found to be closely associated with Uncultured *Pezizaceae* (GenBank accession FJ788766) species isolated from *Pteryqodiumvolucris* plant (58% NJ/ 60% ML /0.62% Mr Bayes) (Fig. 6).

All *Tulasnella* isolates defined morphologically and molecularly were isolated from the roots of *O. coriophora*, *O. laxiflora*, *O. palustris*, *O. papillionaceae*, *O. purpurea*, *O. tridendata*.

Ceratobasidium spp. were isolated from *O. coriophora*, *O. simia* roots and the isolate of *Pezizales* ordo was isolated from *O. laxiflora* roots.

Table 1. Summary of cultural, morphometric characteristics of *Orchis* spp isolates.

Isolate	Fungal taxa	Host	Color of young colony (surface)	Colony appearance	Color of vegetative hyphae	Crystal structure	Diameter of vegetative hyphae (µm)	Nuclear condition
OP 1	*Tulasnellaceae* (Basidiomycota)	*Orchis papillionaceae*	Greyed-Yellow Group	Velvety and submerged	Hyaline	Absence	4,0521	Binucleat
OL 1	*Tulasnellaceae* (Basidiomycota)	*Orchis laxiflora*	Gray- Orange Group	Cotony and submerged	Hyaline	Present	2,844	Binucleat
OC 1	*Ceratobasidiaceae* (Basidiomycota)	*Orchis coriophora*	Yellow White Group	Velvety and submerged	Hyaline	Absence	2,881	Binucleat
OC 2	*Tulasnellaceae* (Basidiomycota)	*Orchis coriophora*	Greyed-Yellow Group	Velvety and submerged	Hyaline	Absence	4,489	Binucleat
OC 3	*Tulasnellaceae* (Basidiomycota)	*Orchis coriophora*	Greyed-Yellow Group	Cotony and submerged	Hyaline	Present	2,830	Binucleat
OS 1	*Ceratobasidiaceae* (Basidiomycota)	*Orchis simia*	Yellow- White Group	Velvety and submerged	Hyaline	Absence	3,174	Binucleat
OPl1	*Tulasnellaceae* (Basidiomycota)	*Orchis palustris*	Greyed-Yellow Group	Velvety and submerged	Hyaline	Absence	4.269	Binucleat
OPr1	*Tulasnellaceae* (Basidiomycota)	*Orchis purpurea*	Greyed-Yellow Group	Velvety and submerged	Hyaline	Absence	3,0346	Binucleat
OT1	*Tulasnellaceae* (Basidiomycota)	*Orchis tridentata*	Greyed-Yellow Group	Velvety and submerged	Hyaline	Present	3.286	Binucleat
OL 2	*Pezizaceae* (Ascomycota)	*Orchis laxiflora*	Gray- Orange Group	Cotony and submerged	Hyaline	Present	10,2098	Multinucleat

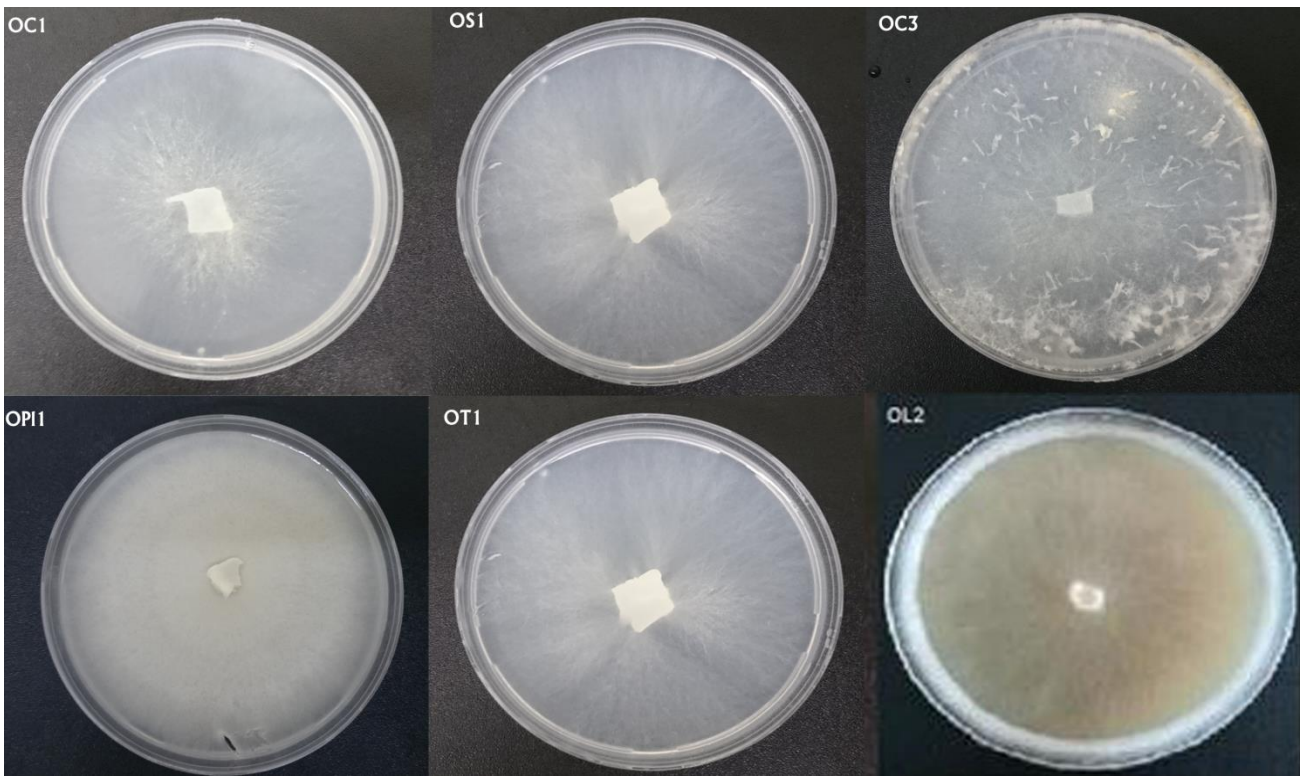

Fig. 2a. Colony appearance of species of Orchis.

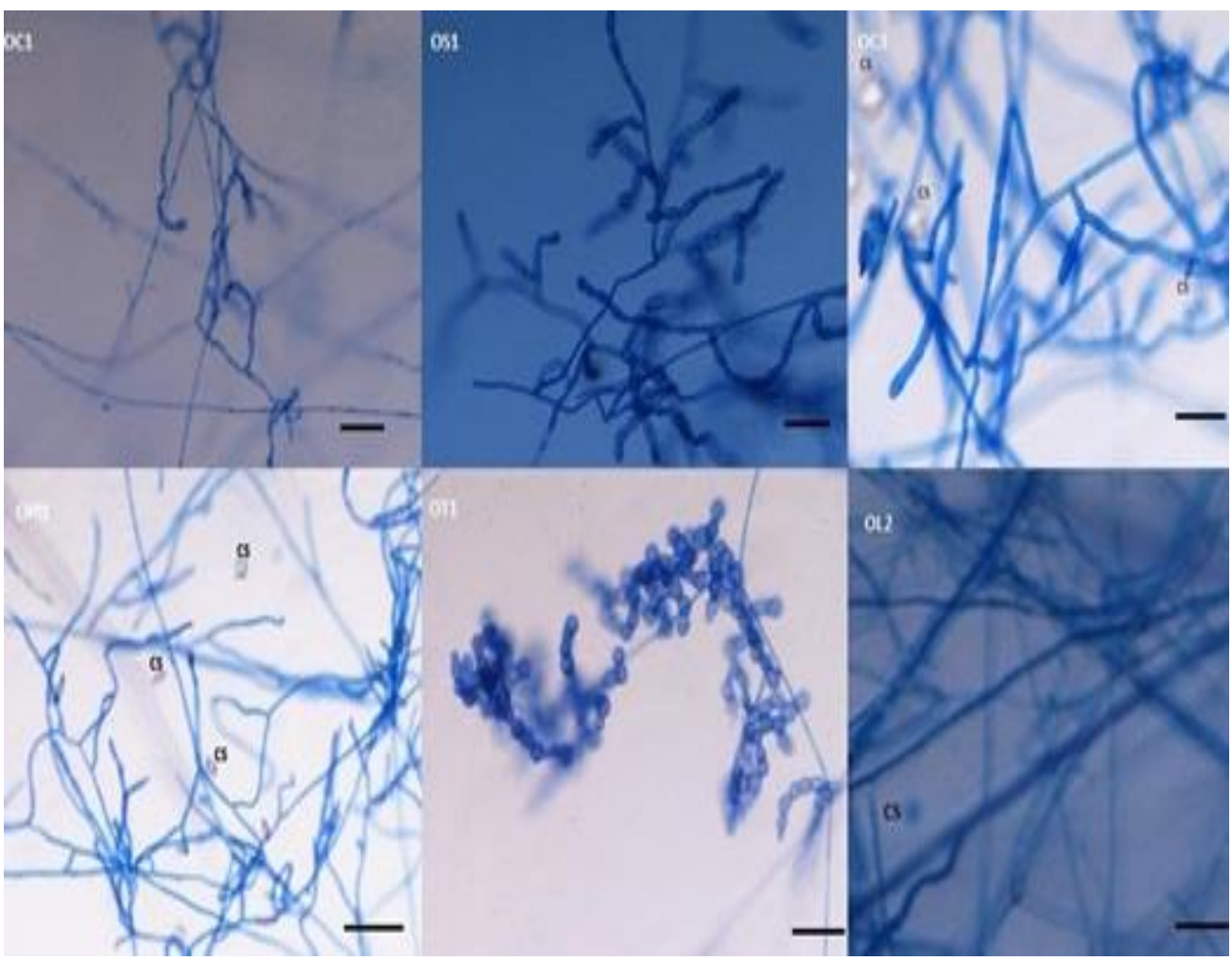

Fig. 2b. Hyphae structure of species of *Orchis* (cs: crystal structure Bar200µm).

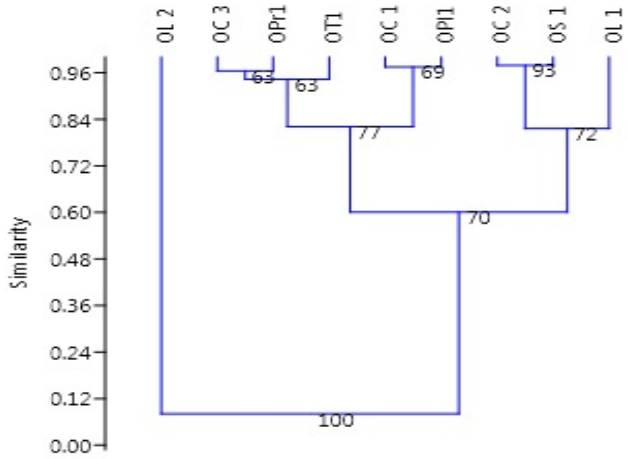

Fig. 3. UPGMA-dendrogram based on morphometric data for six samples of seven *Orchis* species.

Discussion

Turkey is a very rich country with regard to the number of orchid species. Over collection of the bulbs to make salep and ice cream exposes the orchid species to the threat of extinction. The primary factor enabling orchids to propagate from seeds in nature is mycorrhizal fungi. However, detailed studies about the germination of orchids from seeds and mycorrhizal fungal variation are extremely limited in number in Turkey. In Turkey, the morphological and molecular definitions of fungi which join mycorrhizal union in some *Dactylorhiza* species have been conducted (Kompe&Mutlu, 2017); however, there are no studies about the molecular definitions of other species and genus. This study obtained the mycorrhizal fungi of the individuals of 7 species *(O. coriophora, O. laxiflora, O.palustris, O. papillionaceae, O. purpurea, O. simia, O. tridendata)* of *Orchis*genus and their morphological and molecular definitions were made.

In our study, the classifications of isolated fungi according to their morphological features were made based on the study of Sneh *et al.,* (1991). Multivariate analysis was conducted to find out whether the determined morphological variables were effective in species analysis. This analysis was conducted with non-weighted paired group (UPGMA) method and cladogram was constructed. In constructing the cladogram, the nuclei number and colony color of fungal isolates were effective and 3 clusters were formed according to these characteristics (Fig. 3). According to UPGMA cladogram, while *Rhizoctonia*-like fungi of *Orchis* genus were divided in two neighboring groups, non-*Rhizoctonia* group was represented with an isolate which was very far from the others. Non-*Rhizoctonia* OL2 isolate was differentiated from *Rhizoctonia*-like isolates with its multinucleate and grayish yellow colony color as a faraway group. The colony color of binucleate *Rhizoctonia*-like fungi which varied from grayish yellow to whitish yellow was found to be insufficient to discriminate between isolates.

According to the sequence data of fungi reproduced from ITS1-5.8-ITS2 gene area, *Tulasnella* and *Ceratobasidium* (*Rhizoctonia*-like) species and *Pezizaceae* (non-*Rhizoctonia*) family sequences were found to match in NCBI data base. Considering that morphological methods are used commonly in defining fungal isolates (Cruz *et al.,* 2011) and also considering the variation between the cultural characteristics of the same species, it is obligatory to make molecular definitions (Horton & Bruns, 2001; Dernaley *et al.,* 2012). In a similar study conducted in Brazil by Periera *et al.,* (2014), fungi isolated from the roots of *Epidendrum scendrum* could not be differentiated morphologically; however, molecular characterization showed that these isolates were different *Tulasnella* species. This study both found the fungi in the mycorrhizal union of *Orchis* species and tried to get more accurate results through a combined use of morphological and molecular identification methods. Thus, the lacking parts of morphological definitions were completed with molecular methods.

In our study, it was found that the fungi which had mycorrhizal associations with plant roots of *Orchis* species in molecular method based on ITS1-5.8-ITS2 gene area belonged to *Tulasnella*, *Ceratobasidium* genus and *Pezizaceae*family (Table 2). In our study, OL1 and OC3 fungal sequences in *Tulasnella* phylogenetic tree were highly similar to *T.bifrons* (AY373290.1) species isolated from photosynthetic terrestrial orchids of America (McCormick *et al.,* 2004). OP1 and OT1 fungal sequences were found to be associated with the clad which included *T. tomaculum* (KC152380) and *T. violea* (KC152434) species isolated from 7 Equator and 4 Germany orchids and *T. eicleriana* (AY373292) isolated from the photosynthetic terrestrial orchids of America (Cruz *et al.,* 2014; McCormick *et al.,* 2013). While OS1 fungal sequence on the phylogenetic tree of *Ceratobasidium* species was found to be associated with the clad including *C. obscurum* (EU218894) fungal sequence identified in American orchids, OC1 fungal sequence was found to be associated with the clad including *Dactylorhizapurpurella* (HQ680963) species in Germany (Eisold & Grosch, 2010). When the phylogenetic tree of *Pezizacea*ordo was analyzed, OL2 fungal sequence was found to have high resemblance to the fungus of *Pezizaceae* ordo which was isolated from the *Pteyqodiumvolucris*orchid in England and which was uncultured (Waterman *et al.,* 2011).

A lot of researchers have stated that the individuals of *Rhizoctonia* species were with *O. anthropoda, O. mascula, O. militaris, O. purpurea* and *O.simia* (Jacquemyn *et al.,* 2010),*Orchismilitaris* (Sheffersone*t al.,* 2008;Vendramin*et al.,* 2010), *Anacamptislaxiflora, Orchispurpurea, Ophrysfuciflora* and *Serapiasvomeracea* Girlanda *et al.,* (2011) species. In a research by Shefferson *et al.,* (2008), *Epipactisatrorubens* and *O. militaris* species were found to be associated with the fungi of *Tulasnella, Ceratobasidium* and *Pezizales* ordo. The results of our study are in parallel with the results of other studies. In addition, Stark *et al.,* (2009) stated that the fungi of *Pezizaceae* family were also accepted as one of the orchid mycorrhizal fungi. In Turkey, an isolate of Pezizales ordo was obtained from the roots of pink flowered individuals of *Dactylorhizaromana* subsp. *romana* species (Kompe & Mutlu, 2017) and similarly in this study, an isolate was obtained from Pezizaceaeat *O. laxiflora*. It has not been determined exactly whether these isolates encourage germination. In general, it was found that the dominant fungi among the *Rhizoctonia* group fungi found in this study were *Tulasnella* fungi. A great number of other researches have shown that mycobionts of terrestrial or epiphytic orchids were *Tulasnella, Ceratobasidium*, *Sebacina* and /or Pezizales (Taylor *et al.,* 2002; Dearnaley, 2007; Girlanda *et al.,* 2011).

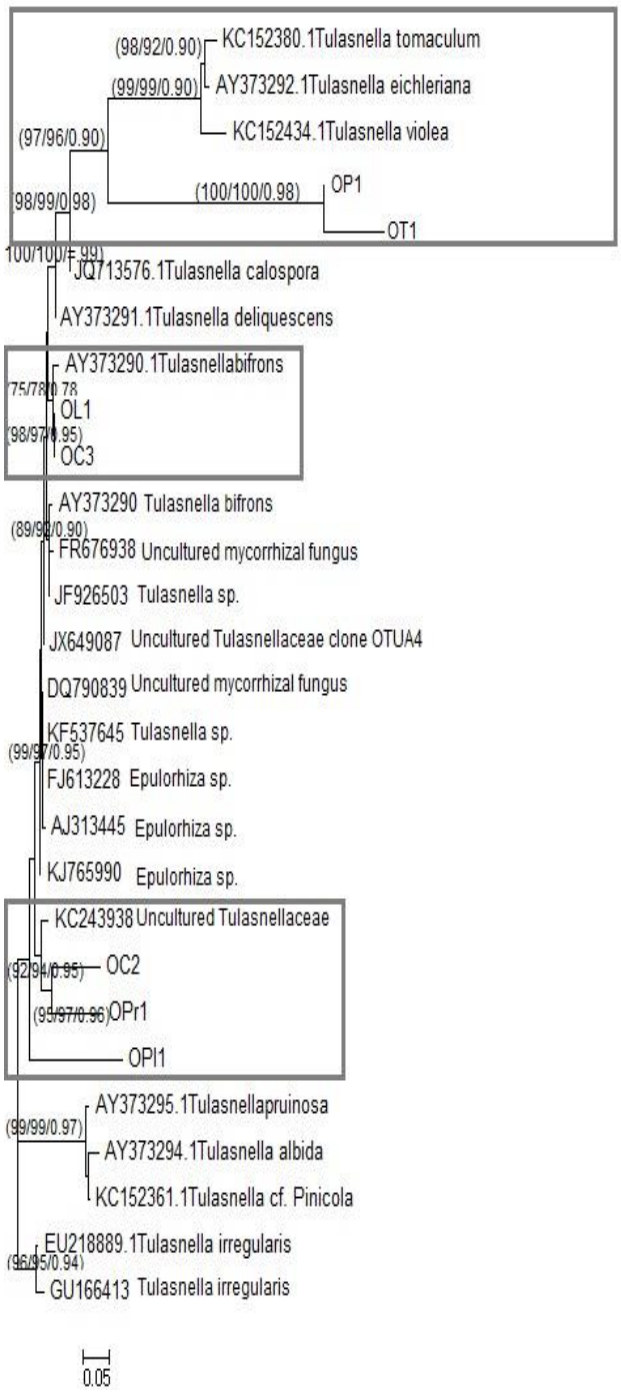

Fig. 4. NJ tree showing the phylogenetic relations of 5.8S rDNA ITS nucleotide sequences of *Tulasnella* genus fungi obtained in this study.On the tree the bootstrap values greater than 50% have been shown and the bootstrap values of NJ, ML and Mr Bayesian trees were stated in paranthesis with this order.

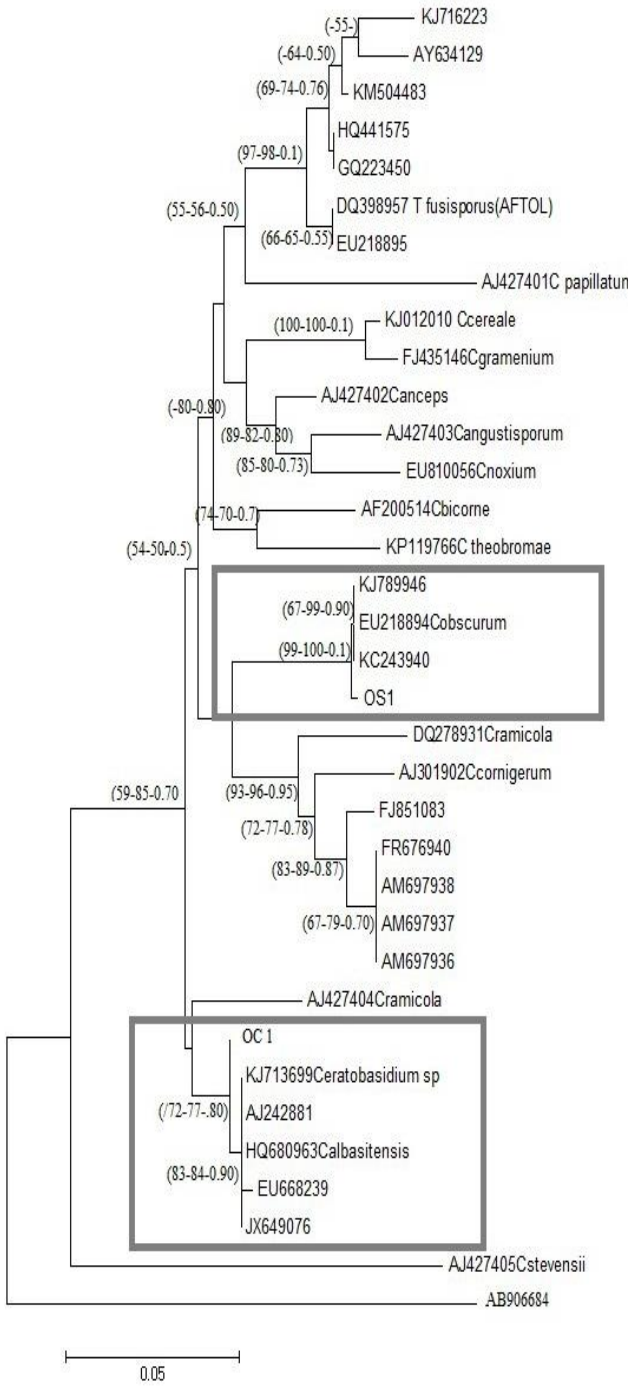

Fig. 5. NJ tree showing the phylogenetic relations of 5.8S rDNA ITS nucleotide sequences of *Ceratobasidium* genus fungi obtained in this study.On the tree the bootstrap values greater than 50% have been shown and the bootstrap values of NJ, ML and Mr Bayesian trees were stated in paranthesis with this order.

In this study, it was found that the fungi of *Tulasnella* species were isolated from orchids spread in different habitats as wet meadow, *Quercus* forest side and dune areas close to the sea. *Ceratobasidium* fungi were isolated from orchids at dune areas and forest sides (*O. coriophora*, *O. simia*, respectively), while Pezizaceae fungi were isolated from orchids at swampland (*O. laxiflora*). Similar results were found in the studies of Jaquemyn *et al.*, (2012) in Belgium, by Girland *et al.*, (2011) in Mediterranean swamp orchids and in the isolations by Kompe & Mutlu (2017) on the roots of *Dactylorhiza* species in Turkey. All these researches have shown that fungi which join orchid mycorrhizal association do not have a specific habitat preference.

This study is the first comprehensive study on the mycorrhizal fungal variety associated with *Orchis* species in Turkey. The content of the first orchid fungus bank generated in Turkey with the isolation and stock of fungi in mycorrhizal union will be extended in future as further studies in this respect will be conducted.

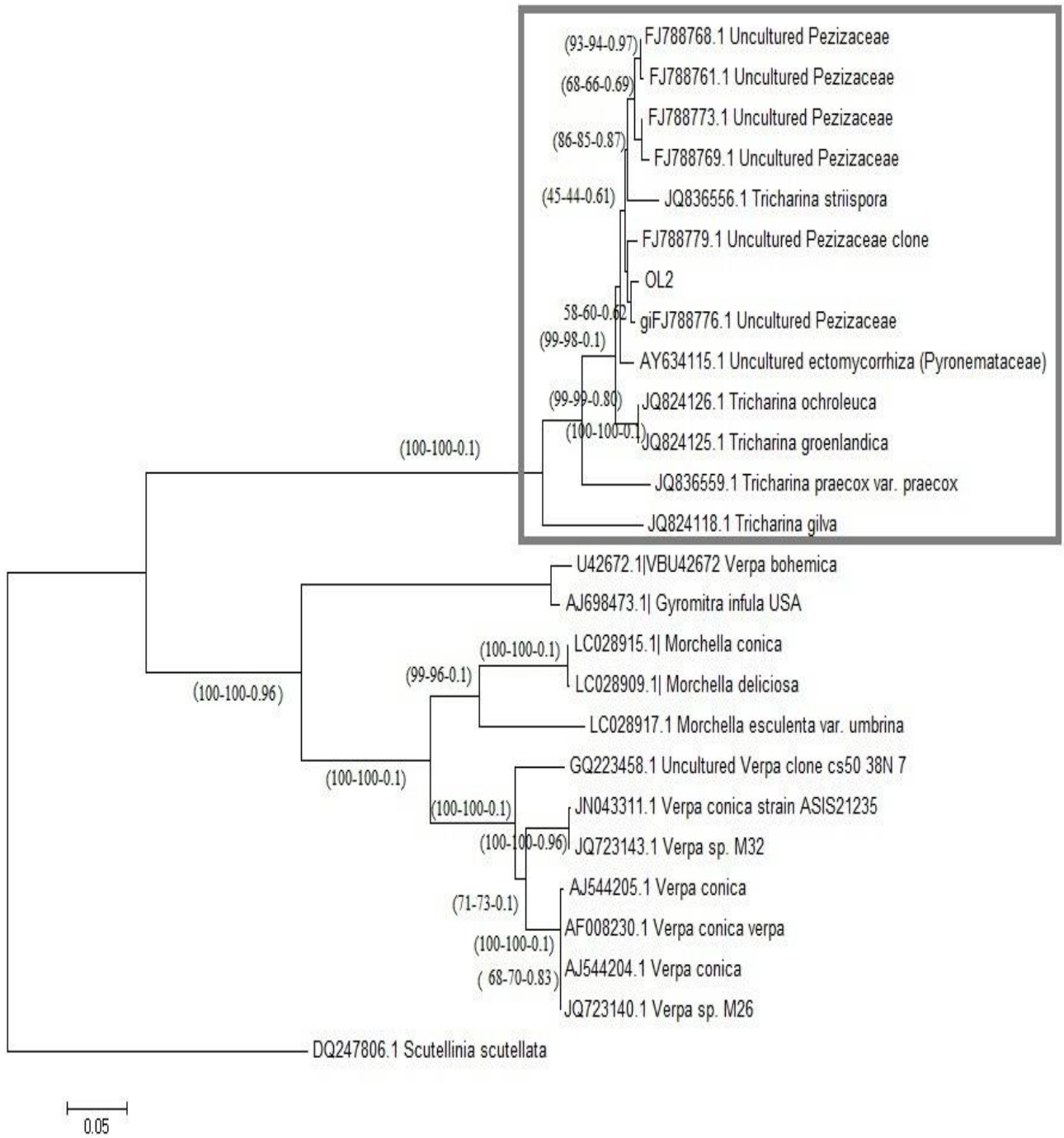

Fig. 6. NJ tree showing the phylogenetic relations of 5.8S rDNA ITS nucleotide sequences of *Peziza* fungi obtained in this study.On the tree the bootstrap values greater than 50% have been shown and the bootstrap values of NJ, ML and Mr Bayesian trees were stated in paranthesis with this order.

Table 2. ITS rDNA sequences analysis of *Orchis* mycorrhizal fungal isolates.

Isolate number	Acession number	Nucleotid (bp)	Description	Identy (%)	Closest match in GenBank (Accession No.)
OP 1	MG762601	624	Uncultured *Tulasnellaceae* clone Di_Aga_3	99	JX024734
OL 1	MG762604	640	Uncultured *Tulasnellaceae* clone VJ4-2A	99	KC243938
OL 2	MG762599	410	Uncultured *Pezizaceae* clone 1211a	99	FJ788779
OC 1	MG762693	484	Uncultured *Ceratobasidiaceae* clone	100	KC243940
OC 2	MG762600	634	Uncultured *Tulasnellaceae* clone Di_Aga_3	99	JX024734
OC 3	MG762598	635	*Tulasnellacalospora*	99	GU166407.1
OS 1	MG762694	479	Uncultured *Ceratobasidiaceae* clone OTUC1_	100	JX649076
OPl1	MG762605	606	Uncultured *Tulasnellaceae* clone Di_Aga_3	99	JX024734
OPr1	MG762603	644	Uncultured *Tulasnella* mycobiont of Aneurapinguis	99	EU909346
OT1	MG762602	603	Uncultured *Tulasnella* clone SV18	100	JF926504.1

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