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Phylogenetic relationships of the genus *Quercus* L. (Fageceae) from three different sections

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In this study, the genetic diversity of 6 oak species known as *Quercus coccifera* L., *Q. robur* L., *Q. infectoria* Oliver, *Q. cerris* L., *Q. ithaburensis* subsp. *macrolepsis* (Kotschy) Hedge and Yalt. and *Q. trojana* P.B. Webb in 18 populations was screened using the randomly amplified polymorphic DNA method (RAPD). 10 RAPD primers giving the best results produced 262 total loci. The highest and lowest band sizes were between 125 and 1800 bp, respectively. The binary RAPD data was computed using the Statistica version 8.0 and Popgene 32, genetic data analysis software program. The principal component analysis and cluster analysis displayed the seperation of populations based on genetic distances. The genetic similarity and distance matrix using Popgene 32 based on Nei (1972) revealed the genetic relations between studied populations. As a result of this study, it may be expressed that genetic relationships are more similar in the species belonging to same section and especially the relationships between *Quercus cerris* and *Quercus trojana* in the section *Cerris* attracts quite attention.

Key words: *Cerris*, genetic relationships, *Quercus*, randomly amplified polymorphic DNA method (RAPD).

INTRODUCTION

Quercus L. (Oak) is a member of family Fagaceae containing very important woody plants (Jawarneh et al., 2013; Alfonso-Corrado et al., 2014). The genus has a natural distribution in the northern hemisiphere in the world with high diversity (Govaerts and Frodin, 1998; Jawarneh et al., 2013; Laakılı et al., 2016). Turkey with 18 species of oaks is an important region with high species diversity (Yaltırık, 1984; Borazan and Babaç, 2003). The classification of the species of *Quercus* by Hedge and Yaltırık (1982), two Turkish authors, has been of great contribution to the research in this field. Before the classification of Hedge and Yaltırık, many intraspecific taxa were classified as species and species concept for *Quercus* taxa was quite narrow (Borazan and Babaç, 2003). Hedge and Yaltırık reduced the total number of *Quercus* taxa from 35 to 18. However, there are still unresolved nomenclatural and typification problems today.

Hybridization has imperative impacts on the enhancement and evolution of numerous plant species (Rieseberg and Ellstrand, 1993; Rieseberg and Wendel, 1993; Arnold, 1997). Furthermore, it can be stated that hybridization behaviour between species of the genus is in high level (Burger, 1975; Van Valen, 1976). Increasing

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Table 1. The locations and populations of the six different oak species used in this study

Population number	Species	Number of sample	Provinces
Pop.1	*Q.ithaburensis*	10	Uşak üniversity/1 Eylül Campüs
Pop.2	*Q.coccifera*	10	Uşak üniversity/1 Eylül Campüs
Pop.3	*Q.robur*	10	Uşak üniversity/1 Eylül Campüs
Pop.4	*Q.trojana*	10	Uşak üniversity/1 Eylül Campüs
Pop.5	*Q.cerris*	10	Uşak üniversity/1 Eylül Campüs
Pop.6	*Q.infectoria*	10	Uşak üniversity/1 Eylül Campüs
Pop.7	*Q.infectoria*	10	The North parts of Banaz
Pop.8	*Q.trojana*	10	Over path of Uşak-Banaz
Pop.9	*Q.cerris*	10	The North parts of Banaz
Pop.10	*Q.ithaburensis*	10	Entry of Banaz
Pop.11	*Q.coccifera*	10	Sivaslı/around of Evrenli park
Pop.12	*Q.ithaburensis*	10	Sivaslı/around of Evrenli park
Pop.13	*Q.trojana*	10	Near to forest managment of Sivaslı
Pop.14	*Q.infectoria*	10	Near to forest managment of Sivaslı
Pop.15	*Q.coccifera*	10	Over path of Ulubey-Eşme
Pop.16	*Q.cerris*	10	Over path of Ulubey-Eşme
Pop.17	*Q.ithaburensis*	10	Over path of Ulubey-Eşme
Pop.18	*Q.infectoria*	10	Over path of Ulubey-Eşme
	Total	180	

evidence supports the presence of hybridization in genomic structure of different taxa that belong to oaks (Kremer et al., 2002; Petit et al., 2004). Therefore, in addition to different plant groups especially the genus *Quercus* has been used to demonstrate the presence of hybridization.

In addition to hybridization, oaks are wind-pollinated species. Generally oak species grow in mixed populations and as a result of this, hybridization is commonly observed among oak species especially in the same group or section (Bacilieri et al., 1996; Borazan and Babaç, 2003; Petit et al., 2004; Charalambos et al., 2011). Hence, taxonomy and systematic relations of the genus are debatable and not clear, despite various morphological, cytogenetic and molecular studies (Williams et al., 1990; Welsh and McClelland, 1991; Yılmaz et al., 2013). Especially multiple molecular techniques such as RAPD have been used in the analysis of hybridization and relative species (Bodenes et al., 1997).

Recently, many different studies are being conducted in order to better understand the genus *Quercus.* For example, studies are being tested on the identification of new and reliable isolation techniques, due to high phenolic content and tannins in leaf samples of *Quercus* (Pandey and Tamta, 2015). However, efforts are made towards the selection of morphological and dendrometric characters for conservation programs and the selection of provenances for reforestation schemes (Laakılı et al., 2016).

In this study, we planned to recognize each studied species and to expose relations between three different sections (*Quercus* L. (white oaks), *Cerris* Loudon (red oaks) and *Ilex* Loudon known as evergreen oaks) members by molecular analysis (RAPD). For this aim, the six species of oaks were used such as *Quercus coccifera* known as evergreen oaks, *Quercus robur* L. and *Quercus infectoria* oliver known as white oaks, *Quercus cerris* L., *Q. ithaburensis* subsp. *macrolepsis* (Kotschy) Hedge and Yalt. and *Q. trojana* P.B. Webb known as red oaks. Furthermore; this study was performed to investigate the genetic diversity of natural populations of the genus *Quercus*.

MATERIALS AND METHODS

Plant materials

Six *Quercus* species containing *Q. coccifera*, *Q. infectoria*, *Q. robur*, *Q. cerris*, *Q. ithaburensis* and *Q. trojana* belonging to three different sections were selected in Uşak, Turkey (Table 1). A representative picture belonging to Campüs region was showed in Figure 1. Study materials consist of the leaves to show variations within and among species. Leaves for statistical analysis were collected from 180 trees for 18 populations. For each population ten tree were selected and for each tree ten young and fresh leaves were collected for molecular study. After that leaf samples were stored on bags with silica gel and for further analysis they were transferred to freezer.

DNA isolation

Genomic DNA was extracted following Nucleospin Plant II Genomic DNA Isolation Kit (MN) protocol (Özbek and Kara, 2013). DNA samples extracted were controlled for the determination of quality before PCR amplification. Isolated DNA was stored in freezer at -20°C.

Figure 1. A representative picture showing the Campus area of study.

RAPD study was performed as a molecular technique (Gonzalez-Rodriguez et al., 2004; Yılmaz et al., 2013). DNA samples of species belonging to each population were amplified using 20 oligonucleotide primers. Primers were evaluated for visible bands, constancy, and unambiguity. Satisfactory results were obtained from ten of these primers which gave reproducible amplification products. RAPD primers which cause difficulty to detect band and faint were not used for amplification.

RAPD assays for DNA amplification were performed in total volume of 25 µl containing 10 ng of genomic DNA, 1x PCR buffer, 3 mM of $MgCl_2$, 0.36 µM 10-mer RAPD primer, 100 µM dATP, dCTP, dGTP, dTTP and 1.0 unit taq DNA polymerase. The thermal cycling program was obtained as follows; preliminary 94°C for 3 min, 40 cycles of 94°C for 1 min, 32°C for 1 and 72°C for 2 min. After the 40 cycle, an additional final 10 min extension at 72°C was used to complate amplification reactions. The PCR products were analysed by electrophoresis on 1.5% agarose gels with TAE buffer, visualized by ethidium bromide staining, and photographed under UV light. 100 bp plus DNA ladder (Thermo) was used to estimate the molecular weights of amplified fragments.

Data analysis

In the data analysis of PCR products, whereas presence of each band was coded as 1, absence of band was coded as 0 in all individuals. Only clear and score able bands were evaluated for molecular diversity analysis. The analysis of molecular variance was calculated using the percentage of polymorphic fragments within and among species studied.

The bivariate (1/0) data were used to estimate genetic similarity and genetic distance described by Nei (1972) using computer program Pop Gene 32. Cluster analysis (CA) and Principal component analysis (PCA) with arithmetic averages (UPGMA) were performed to show polymorphism among populations belonging to each species.

RESULTS

The surveyed RAPD primers produced 262 total bands. Molecular sizes of amplified fragments ranged from approximately 125 to 1800 bp (Table 2). During present study, a total of 262 DNA fragments were amplified in 180 individuals representing 18 populations using 10 RAPD primers sets. It is showed in Table 2 that maximum amplicons with 30 were generated by primer OPC-06; whereas OPS-09 primer amplified 23 bands. Highest band size with 1800 bp was amplified with OPS-09 primer, while lowest band size (125 bp) was produced with OPX-04. The range of polymorphism ranged from 96.2 to 100% (Table 2).

It can be observed in Table 2 that the list and sequence of primers, the size range observed in 18 populations and percentage of polymorphism for each primer.

In order to score the PCR amplification fragments, each population were run separately with 10 primers. Bands having same mobility in the length were considered as identical fragments. An example of PCR amplification profile obtained from RAPD primer OPC-09 is presented in Figure 2. The bivariate data (1/0) and dissimilarity coefficient matrices of 18 populations of 6 *Quercus* species based on the data of 10 RAPD primers were used to construct separate dendrograms using statistica version 8.0 and popgene 32 (Figures 3 and 4 and Table 3).

Cluster analysis and principal component analysis were carried out to show variations among studied species and to group of populations belonging to 6 oak species.

Table 2. Name, sequence, number of bands provided from RAPD primers and size range observed in 18 populations of the genus *Quercus.*

Primers	Sequence (5'-3')	Number of bands	Number of polymorphic bands	Size Range (bp)	Percentage of polymorphism
OPA-01	CAGGCCCTTC	29	29	200-1600	100
OPA-03	AGTCAGCCAC	27	26	150-1500	96.2
OPA-07	GAAACGGGTG	25	25	150-1500	100
OPA-13	CAGCACCCAC	24	24	150-1200	100
OPX-04	CCGCTACCGA	27	27	**125**-1600	100
OPC-06	GAACGGACTC	30	30	150-1600	100
OPU-01	ACGGACGTCA	27	26	150-1500	96.2
OPC-09	CTCACCGTCC	25	25	200-1500	100
OPS-09	TCCTGGTCCC	23	23	200-**1800**	100
OPS-20	TCTGGACGGA	25	25	150-1500	100

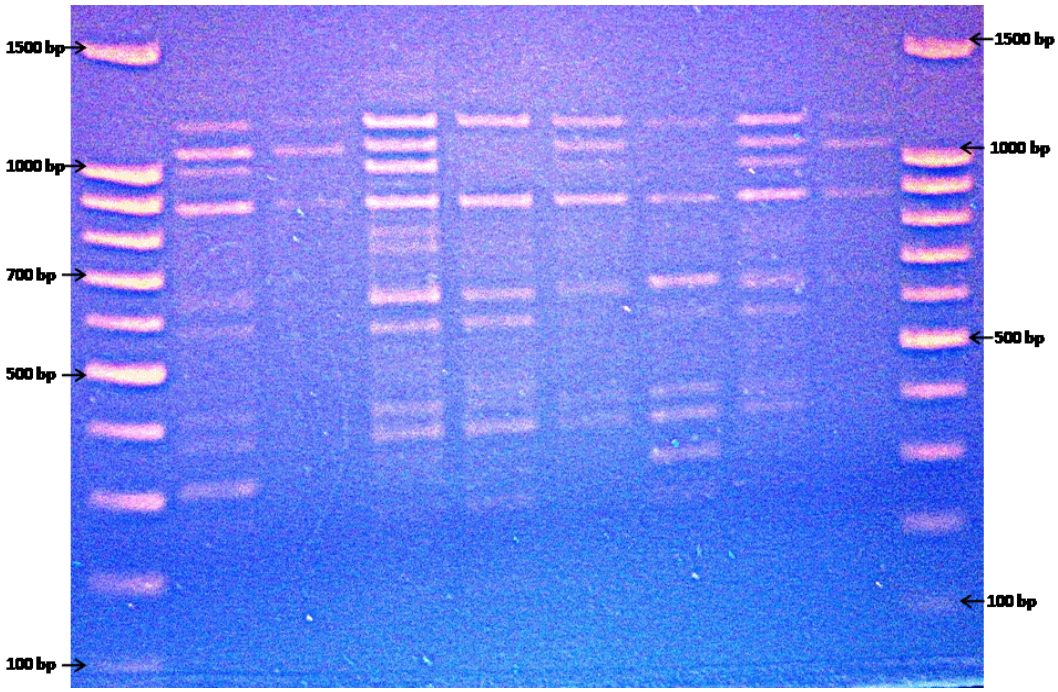

Figure 2. The representative figures of *Quercus infectoria* (Pop. 6) with OPC-09 RAPD-PCR band patterns.

Based on the analysis carried out on PCA, it can be said that the populations belonging to same species were generally observed within same group, in other words studied species were separated from each other (Figure 3).

In Figure 4, dendrogram generated by UPGMA cluster analysis of RAPD fragments indicate that the genotypes were grouped in 8 main groups (A, B, C, D, E, F, G and H).

The largest group was Group D comprising of 4 genotypes and group B and G consist of 3 genotypes each. Group C, F and H consisted of 2 genotypes. Group A and E were smallest and comprised of only 1 genotype.

The genetic similarity and genetic distance matrix derived from RAPD data using popgen 32 (Nei, 1972) are presented in Table 3. According to the results provided from Table 3, the lowest genetic distance was determined between population 4 and population 13. These are two populations belonging to *Q. trojana* species from different regions. Same results can be observed in the Figures 3 and 4 obtained from CA and PCA. The highest genetic distance was between populations 3 to 17 and populations 6 to 17 belonging to two genetically distant taxa, respectively.

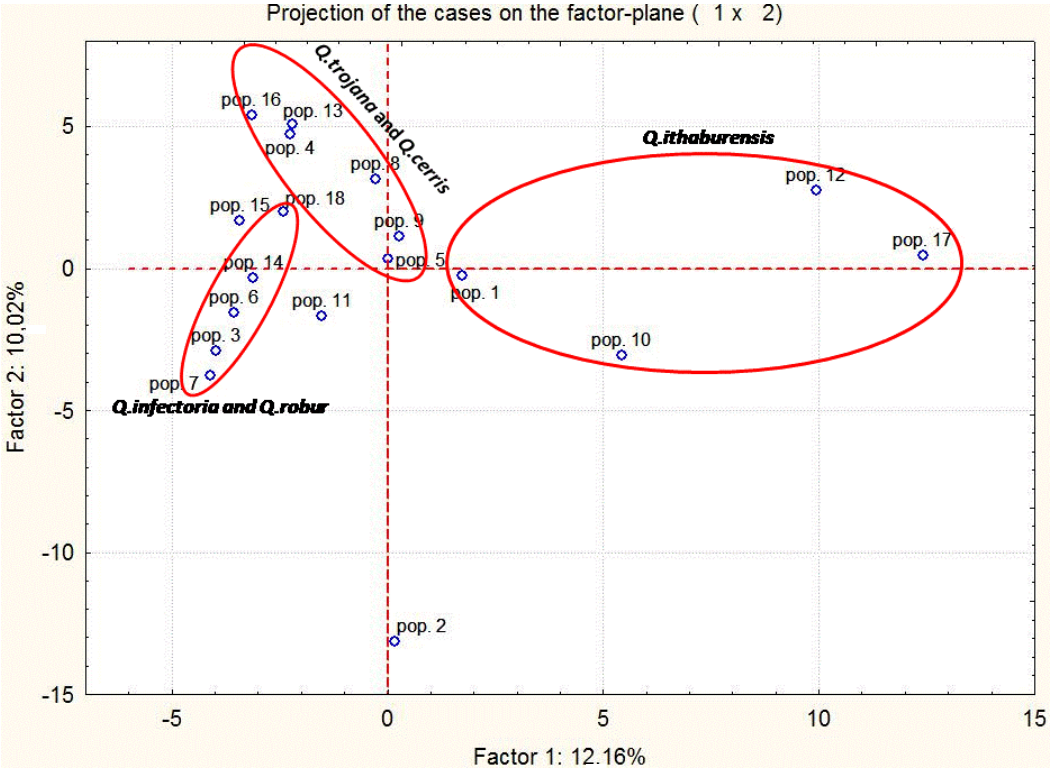

Figure 3. The resulting projection of principal component analysis.

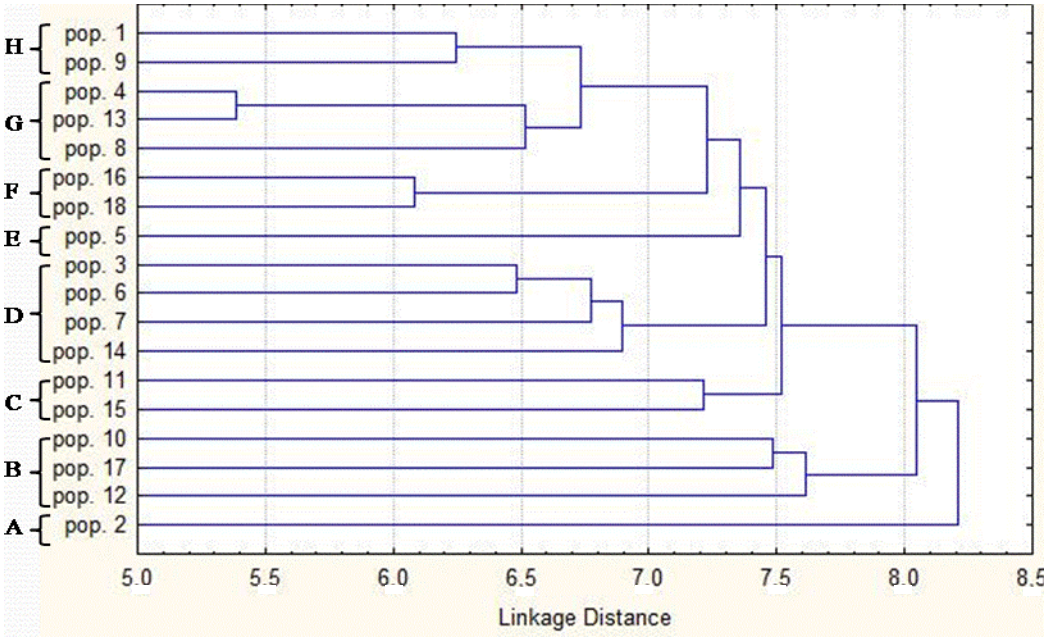

Figure 4. A dendrogram representing phylogenetic relationships between 18 *Quercus* populations.

DISCUSSION

Different DNA markers are widely used to reveal polymorphism within and among plant species (Gonzalez-Rodriguez et al., 2004; Yu et al., 2005; Coelho et al., 2006; Faltusova et al., 2011; Ardi et al., 2012). Recently,

Table 3. The compution of genetic similarity (upper diagonal) and genetic distance (below diagonal) (Nei, 1972).

Pop ID	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	****	0.993	0.981	0.965	0.992	0.984	0.976	0.982	0.984	0.986	0.986	0.974	0.975	0.984	0.961	0.960	0.961	0.982
2	0.007	****	0.980	0.948	0.974	0.981	0.980	0.969	0.974	0.983	0.982	0.959	0.957	0.976	0.955	0.941	0.936	0.967
3	0.019	0.020	****	0.977	0.972	0.992	0.979	0.989	0.994	0.984	0.980	0.982	0.944	0.984	0.969	0.971	0.923	0.981
4	0.035	0.053	0.022	****	0.980	0.982	0.970	0.984	0.992	0.962	0.964	0.955	**0.997**	0.990	0.981	0.937	0.991	0.987
5	0.007	0.025	0.028	0.019	****	0.978	0.967	0.981	0.987	0.975	0.977	0.980	0.986	0.988	0.967	0.964	0.978	0.985
6	0.015	0.018	0.007	0.017	0.021	****	0.991	0.989	0.992	0.979	0.973	0.947	0.985	0.988	0.969	0.928	0.966	0.987
7	0.023	0.020	0.020	0.029	0.032	0.008	****	0.984	0.981	0.981	0.975	0.956	0.977	0.981	0.961	0.929	0.951	0.971
8	0.018	0.030	0.011	0.015	0.018	0.010	0.016	****	0.995	0.990	0.982	0.965	0.993	0.984	0.962	0.944	0.982	0.985
9	0.015	0.026	0.005	0.007	0.013	0.007	0.018	0.005	****	0.983	0.981	0.962	0.995	0.992	0.977	0.940	0.987	0.989
10	0.013	0.016	0.015	0.037	0.025	0.020	0.019	0.010	0.016	****	0.994	0.975	0.976	0.978	0.958	0.960	0.962	0.975
11	0.014	0.018	0.019	0.036	0.023	0.027	0.024	0.017	0.018	0.005	****	0.981	0.974	0.984	0.973	0.972	0.967	0.978
12	0.025	0.041	0.056	0.046	0.019	0.053	0.044	0.035	0.038	0.024	0.018	****	0.966	0.970	0.953	0.985	0.957	0.960
13	0.025	0.043	0.018	0.002	0.013	0.014	0.022	0.006	0.004	0.024	0.025	0.034	****	0.990	0.975	0.946	0.993	0.988
14	0.015	0.023	0.015	0.009	0.011	0.011	0.018	0.015	0.007	0.021	0.015	0.030	0.009	****	0.992	0.960	0.982	0.994
15	0.038	0.045	0.031	0.018	0.032	0.030	0.039	0.038	0.023	0.042	0.027	0.047	0.024	0.007	****	0.953	0.974	0.983
16	0.039	0.066	0.029	0.008	0.021	0.034	0.050	0.018	0.012	0.038	0.033	0.043	0.006	0.017	0.026	0.945	****	0.983
17	0.040	0.060	**0.079**	0.065	0.036	0.074	0.072	0.056	0.060	0.040	0.027	0.014	0.054	0.040	0.047	****	0.056	0.961
18	0.017	0.032	0.019	0.012	0.014	0.011	0.028	0.014	0.010	0.024	0.022	0.040	0.011	0.006	0.016	0.039	0.017	****

different PCR based techniques such as amplified fragment length polymorphism (AFLP), random amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP), single nucleotide polymorphism (SNP), simple sequence repeats (SSR) are used to determine the genetic variations and diversity at molecular level (Bruschi et al., 2003; Coelho et al., 2006; Franjic et al., 2006; Yılmaz et al., 2013). Despite the short-comings like problems related to reproducibility in amplification of RAPD markers, inadequacy to distinguish between heterozygotes and homo-zygotes, the RAPD method having advantages such as the high level of polymorphism and simply applicability was prefered as molecular technique to evaluate 6 oak species.

The results show that the method could reveal the genetic relationship among six species of oaks and distinguish them. Here studied species are represented by 3 different sections such as *Q. robur* and *Q. infectoria* in *Quercus* section, *Q. coccifera* in *Ilex* section and *Q. cerris*, *Q. ithaburensis* and *Q. trojana* in *Cerris* section. In this study, the molecular analysis with CA and PCA revealed a high degree of separation between studied species. According to dendrogram derived from CA, populations were grouped in 8 main groups (A, B, C, D, E, F, G and H). Generally populations of same species are localized in same group. For example, group B consists of the populations of *Q. ithaburensis*, group C from populations of *Q.coccifera*, group D from populations of *Q.infectoria* and *Q. robur*, group G from the populations of *Q. trojana*. The separation of species can be clearly observed in Figures 3 and 4.

When the dendrogram which consists of 8 main groups was investigated; it can be stated that *Q. infectoria* and *Q. robur* were observed as close species within same main group (group D) (Figures 3 and 4). These are two species belonging to section *Quercus*. Populations of *Q. coccifera* that belong to section *Ilex* showed more differences in comparison to other studied species (group A and C). Finally; the relationships among the species of the section *Cerris* draws attention, especially between *Q. cerris* and *Q. trojana*. The table of genetic similarity and distance supports this situation (Table 3).

When the distribution of populations according to dendrogram obtained from CA and PCA is

investigated, it is observed that there is more similarity in species belonging to same section and the relationship among species of section *Cerris* attracts quite attention.

As a result of this study, it may be expressed that the molecular analysis disclosed useful results in explaning genetic diversity within and among populations belonging to six species from three different section. Furthermore; study results provide quite contribution to understanding the relationships among the sections of the genus *Quercus*, as well as interactions betweeen species studied.

Conflict of Interests

The authors have not declared any conflict of interests.

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