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Original article

# Genetic Diversity of Some *Quercus* (Fagaceae) and their Putative Hybrids in Turkey

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**Abstract**

In the study, Inter-Simple Sequence Repeat (ISSR) method was used to identify and differentiate between twelve different white oaks to show their genetic diversity. On the other hand, interspecific hybridization is quite common among oak species. This situation makes the hybridization between closely related parents possible. Besides genetic diversity of some white oaks, the five putative hybrids which are morphologically indistinguishable were also studied. ISSR markers produced a total of 89.71 % polymorphism with Quercus taxa and a total of 175 bands were revealed by 11 ISSR primers. Statistical analysis software's, Minitab, NTSYS-pc (Numerical Taxonomy and Multivariate Analysis System) and POPGENE (Population Genetic Analysis) software's were used to reveal variations between these white oaks. Effective allelic frequency, Shannon index, genetic distance was calculated by the POPGENE software. The most distance taxon was *Q. pontica*, then *Q. vulcanica* found to be genetically distant among the taxa. The results of the two analyses, cluster (CA) and principal component (PCA) are in correlation with each other and giving four groups among the studied oak taxa. Putative hybrids are usually located between their presumed parents in the dendrogram and graphs. Consequently, this preliminary study showed that ISSR markers can be used with confidence for genetic diversity of white oaks. It can also be helpful for putative hybrids to some extent.

**Keywords:** **H**ybridization, ISSR, PCA, Polymorphism, White Oaks.

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## INTRODUCTION

*Quercus* is the most important and the largest genus of Fagaceae in terms of species diversity, as well as ecological dominance (Nixon, 2008). It includes about 531 species, distributed in America, Asia, Eurasia, North Africa, and the tropical region of the Northern Hemisphere (Govaerts & Frodin, 1998). High levels of genetic variations, which is another attracting feature of oaks, are observed within and among species (Kremer & Petit 1993; Curtu et al., 2007*a, b*).

Interspecific hybridization is the quite common phenomena among oaks, due to poor reproductive isolation mechanisms among closely related taxa that often occur in mixed stands (Ortego & Bonal, 2010). Although hybridization occurs between species in the same group, a few reports of such crosses exist between different groups (Nixon, 2008).

Genus, *Quercus*, is economically quite important for Turkey and has been used as timber, and coppice. The nuts of oaks are also used for erosion control (Borelli & Varela, 2000). About one-third of the total forest area is covered with oaks in Turkey (Çolak & Rotherham, 2006). The genus is represented by 24 taxa under the following 3 sections: *Quercus* (white oak), *Cerris* (red oak), and *lIex* (evergreen oak) (Hedge & Yaltırık, 1982; Yaltırık, 1984). Recently, a new subspecies, *Q. trojana* subsp. *yaltirikii* was also added to this number (Zielioski et al., 2006). Additionally, 31 hybrids have been recognized in Turkey due to interspecific hybridization (Kasaplıgil, 1992).

Although there have been many studies on classification of oak taxa in Europe and the Middle East (Camus, 1934–1954; Kotschy, 1858–1862; Menitsky, 1984; Schwarz, 1937; Zohary, 1966), *Quercus* is still one of the most taxonomically and genetically questionable woody group in Turkey and the world. These problems result from the interspecific variations of oaks, especially based on morphological characters. First of all, a reliable diagnosis can be made when both leaf and fruit characters are together in oak diagnosis. If there is no fruit, diagnosis from leaves is often not possible. If someone takes the leaf sizes, many taxa can be in the same size range as each other. For example, *Q. petraea, Q. infectoria, Q. frainetto, Q. pubescens* cannot be distinguished according to their leaf size. Leaf shape of all the taxa is generally inverted egg-shaped, and the sizes are 6-17 X 3-9 cm for *Q. petraea*, 4-7-10 X 1.0 -5.0 cm for *Q. infectoria*, 20 X 13 cm for *Q. frainetto* and 4.5-8.5 X 2.5-5.0 cm for *Q. pubescens.* Especially for *Q. infectoria* leaf size and colour cannot be reliable as it is highly variable. Because, the continuous variation is observed in these traits, especially among individuals from mixed stands. Even these ranges closer to each other between the subspecies. A similar situation is observed for fruit sizes and shapes (Hedge &Yaltırık, 1982).

Despite the high number of studies, the genus *Quercus* is still considered a "difficult" group for taxonomists. The recognition of parental species and their hybrids in the wild is usually difficult due to the lack of clearly diagnostic leaf morphological markers. For this reason, molecular studies are

providing useful diagnosis and increasing the reliability for classification and understanding their genetic background. Gene flow indication between oaks taxa was first published on the basis of shared patterns of chloroplast DNA haplotypes of white oaks (Whittemore & Schaal, 1991, Manos et al., 1999). Microsatellite (SSR) markers were also used to analyze the genetic structure and genetic diversity of many Fagaceae members and others (Aldrich et al., 2005; Chokchaichamnankit et al., 2008; Coutinho et al., 2014; Gamar et al., 2018; Rahmat et al., 2019). ISSR markers are universal, quick, easy to apply, highly reproducible, polymorphous, and useful to study inter/intra-specific relationships. These markers are also useful to identify and characterize oak hybrids (Bornet & Branchard, 2001; Ishida et al., 2003; James & Abbot, 2005; Carvalho et al., 2009). ISSR markers were also used for identification of bread wheat genomic regions which conferring drought tolerance (Maqsood et al., 2017). In another study, genetic diversity, and positions of seven endangered *Camellia chekiangoleosa* Hu populations were identified by ISSR (Xie et al., 2018). ISSR markers were also used similarly in some other species (Amine et al., 2014; Ding et al., 2016; Oğraş et al., 2017; Guliyev et al., 2018).

In this study, white oaks and their five putative hybrids from Turkey, (*Q. infectoria* Oliver subsp. *infectoria* × *Q. petraea* Liebl subsp. *iberica* Krassiln., *Q. petraea* subsp. *iberica* × *Q. frainetto* Ten., *Q. macranthera* Fisch&Mey.ex Hohen × *Q. pubescens* Willd, *Q. infectoria* × *Q. pubescens*, *Q. virgiliana* Ten × *Q. pubescens*) were used for identification and differentiation. There are many studies on Turkish *Quercus*, but little information is available with regard to its genetics and their hybrids. Therefore, the aim of this research was to study the genetic diversity of white oaks and to try to reveal putative hybrids with their possible parents with the help of ISSR markers.

## MATERIAL and METHODS

### Plant material

In this study, 12 different white oaks and 5 putative hybrids, making a total of 35 white oak individuals were used, the list of taxa is given in Table 1. Repeats of the same taxon, were presenting different localities. Hybrids and oaks were try to be identified based on the morphological characters of *Quercus* in Turkey (Hedge & Yaltırık, 1982; Yaltırık, 1984).

All specimens were collected in earlier expeditions and kept in AIBU herbarium (Bolu, Turkey). Geographical distributions of studied materials are presented in Figure1.

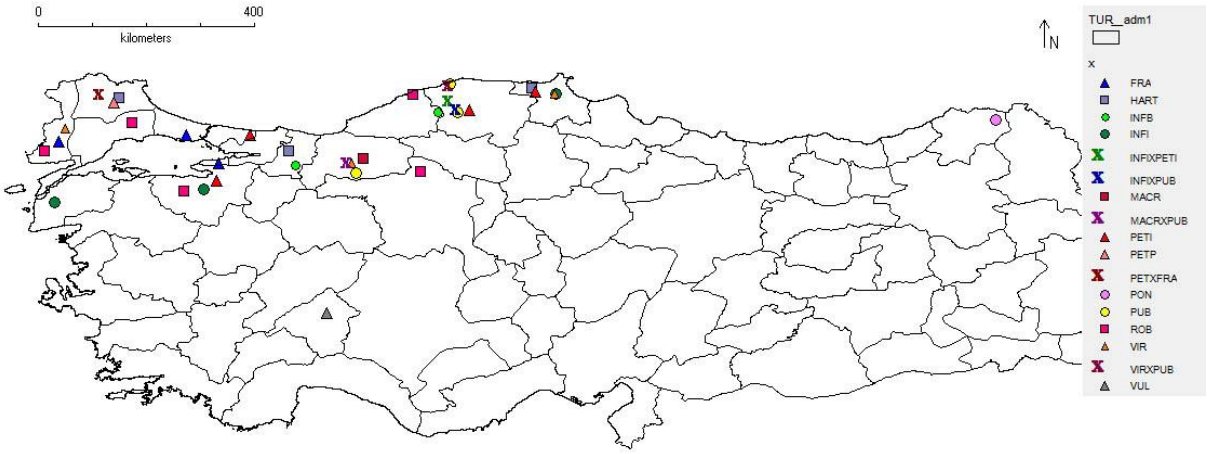


**Figure 1.** Distributions of the collected oak materials. The symbol refer to codes of used species *(the whole name of these codes were given in Table 1).*

**Table 1.** Studied *Quercus* taxa list, their scientific names, localities, and abbreviations.

| Sample No | Taxon name | Localities in order of samples | Figure Abbreviation |
|---|---|---|---|
| 1-3 | *Q.frainetto* Ten | İstanbul, Edirne, Yalova | Fra |
| 4 | *Q.petraea* Liebl subsp. *iberica* Krassiln.× *Q.frainetto* | Kırklareli | Peti×Fra* |
| 5-8 | *Q.petraea* Liebl subsp. *iberica* Krassiln. | İstanbul, Sinop, Bursa, Kastamonu | Peti |
| 9 | *Q.petraea* Liebl subsp. *petraea* | Kırklareli | Petp |
| 10 | *Q.infectoria* Olivier × *Q.petraea* Liebl subsp. *iberica* Krassiln. | Sinop | Infi×Peti* |
| 11-12 | *Q.infectoria* Olivier subsp. *boissieri* O. Schwarz | Sakarya, Kastamonu-Daday | Infb |
| 13-15 | *Q. infectoria* Olivier subsp. *infectoria* | Sinop, Bursa, Çanakkale | Infi |
| 16 | *Q. vulcanica* Kotschy | Isparta | Vul |
| 17 | *Q. pontica* C. Koch | Artvin | Pon |
| 18-20 | *Q. pubescens* Willd | Kastamonu-Cide, Eflani, Bolu, | Pub |
| 21 | *Q.virgiliana* Ten × *Q.pubescens* Willd | Kastamonu | Vir×Pub* |
| 22 | *Q.infectoria* Olivier × *Q.pubescens*Willd | Kastamonu-Eflani | Inf×Pub* |
| 23-25 | *Q.virgiliana* Ten | Sinop, Bolu, Edirne | Vir |
| 26 | *Q.macranthera* Fisch & Mey.ex Hohen subsp. *sypirensis* Menitsky | Bolu | Macs |
| 27 | *Q.macranthera* × *Q.pubescens* Willd | Bolu | Macs×Pub* |
| 28-32 | *Q. robur* L. subsp *robur* | Ankara, Tekirdağ, Edirne, Bursa, Bartın | Robr |
| 33-35 | *Q. hartwissiana* Steven | Sinop, Sakarya, Kırklareli | Hart |

(*Possible putative hybrid taxa)

**DNA extraction and PCR analysis**

The genomic DNA was extracted from silica gel dried leaf samples by the hexadecyltrimethylammonium bromide (CTAB) method (Doyle & Doyle, 1990). Copying of template DNA was achieved in a Techne $^{3}$Prime Thermal Cycler. For Polymerase Chain reaction (PCR) analysis, 20 ISSR markers were tested and 11 were found to be suitable. Their list is given in Table 2.

PCR amplification reactions were operated in a total volume of 30 µl consisting of 25 ng total genomic DNA, 10 µM dNTPs, 10 µM primer, 25 mM $MgCl_2$, 10× buffer, and 1 U *Taq* polymerase (Thermo Scientific) (Carvalho et al., 2005).

Water was used instead of DNA for negative control. The amplified products were separated by 1.7 % agarose gel electrophoresis in 1 X TBE buffer and stained with 0.5 µg/mL ethidium bromide. The gel was viewed using UV light and photographed. ISSR-PCR amplified products were loaded on the agarose gel in a particular arrangement such that the putative hybrid samples were run between their potential parents as indicated by vertical arrows (Figure 2).

**Data analysis of ISSR**

All fragments obtained by 11 primers with 35 samples were scored manually. Each reaction was duplicated, and only reproducible bands were examined for analysis. The band sizes were determined based on Mass Ruler DNA ladder (Thermo Fisher) and were coded for absence (0) or presence (1). The genetic similarity between studied taxa was determined according to Nei's genetic distance coefficient (Nei, 1987). Unweighted pair group (UPGMA) dendrogram was constructed using NTSYSpc software, while Principal Component Analysis (PCA) was performed by Minitab. Genetic variations of all loci, PIC values, Shannon Index and Genetic distance were calculated by POPGENE 1.32 software.

## RESULT and DISCUSSION

ISSR markers showed sufficient polymorphism (89.71 %) with *Quercus* taxa; a total of 175 bands were revealed by 11 ISSR primers. Assuming a Hardy–Weinberg equilibrium, the effective number of alleles per locus (ne) ranged from 1.3243 to 1.6020, with an average of 1.4728; Nei's gene diversity (h) ranged from 0.2097 to 0.3582, with an average of 0.2813; Shannon's information index (I) ranged from 0.3312 to 0.5374, with an average of 0.4283 (Table 2).

**Table 2.** Genetic diversity of studied populations and *Quercus* taxa based on ISSR

| **Loci** | **Sample Size** | **na*** | **ne*** | **h*** | **I*** |
|---|---|---|---|---|---|
| 807(100-1000) | 35 | 2.0000 | 1.4683 | 0.2768 | 0.4422 |
| 817(150-1500) | 35 | 2.0000 | 1.3593 | 0.2186 | 0.3467 |
| 825 (200-3000) | 35 | 2.0000 | 1.6020 | 0.3582 | 0.5374 |
| 841 (150-1000) | 35 | 2.0000 | 1.4175 | 0.2654 | 0.4155 |
| 835 (200-1100) | 35 | 2.0000 | 1.3973 | 0.2451 | 0.3775 |
| 850 (150-1500) | 35 | 2.0000 | 1.5098 | 0.2952 | 0.4451 |
| 856 (200-1500) | 35 | 2.0000 | 1.3243 | 0.2097 | 0.3312 |
| 823 (200-1700) | 35 | 2.0000 | 1.5854 | 0.3304 | 0.4931 |
| 878 (250-3000) | 35 | 2.0000 | 1.5688 | 0.3233 | 0.4814 |
| 846 (100-1500) | 35 | 2.0000 | 1.3800 | 0.2358 | 0.3660 |
| 826 (250-3000) | 35 | 2.0000 | 1.5448 | 0.3178 | 0.4736 |

* na = Observed number of alleles

* ne = Effective number of alleles

* h = Nei's (1973) gene diversity

* I = Shannon's Information index

An example of ISSR marker, UBC 878 is given in Fig. 2. In the figure, horizontal arrows indicate common bands between putative hybrids and in their possible parents, and the vertical arrows specify putative hybrids.

According to Nei's genetic distance similarity, a dendrogram was obtained by NTSYSpc software, which shows four clusters at about 0.44 coefficient level (Figure 3). Cluster I included *Q. petraea* subsp. *iberica* and *Q. frainetto* individuals, and their putative hybrid *Q. petraea* subsp. *iberic*a × *Q. frainetto*.

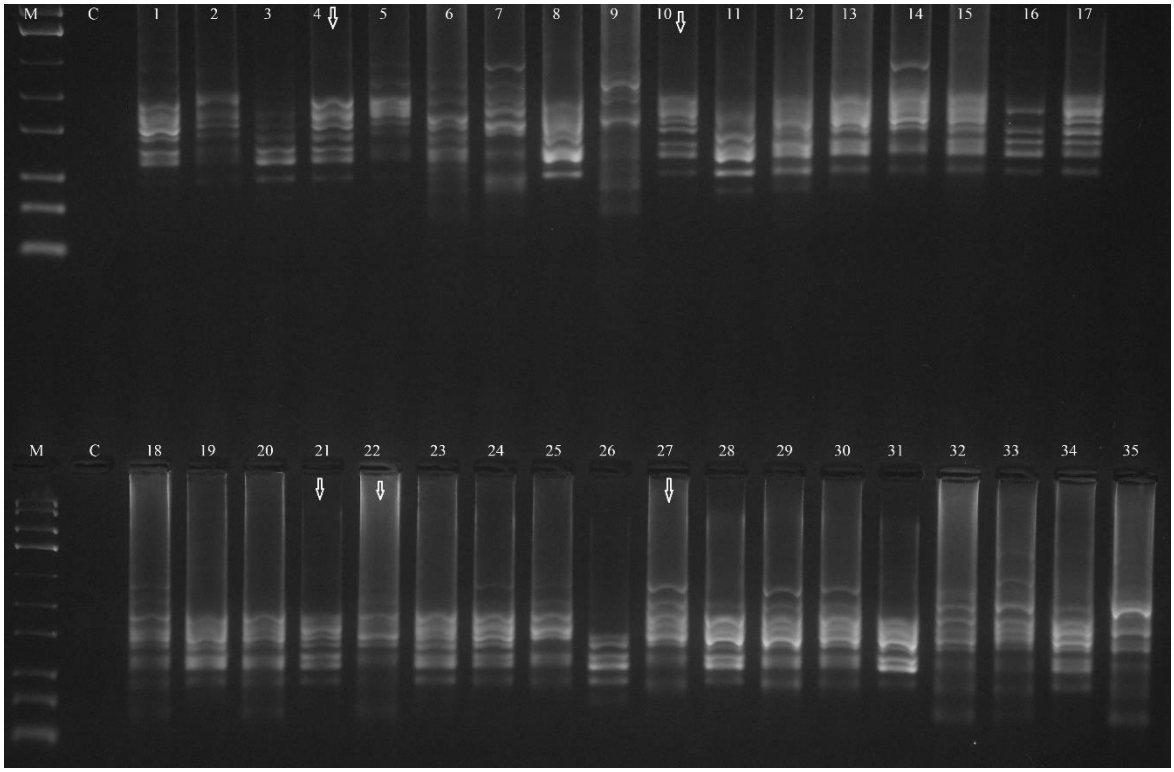


**Figure 2.** Amplified products by the UBC 878 primer in the 35 oak individuals.

M : Mass Ruler DNA ladder mix C : negative control, Samples 1,2,3- (Fra), 4- (Peti×Fra), 5-7,9- (Peti), 8- (Petp),10- (Infi×Peti), 11,12- (Infb), 13-15- (Infi),16- (Vul),17- (Pon), 18-20- (Pub), 21- (Vir×Pub), 22- (Infi×Pub), 23-25- (Vir), 26- (Macs), 27- (Macs×Pub), 28-32- (Robr), 33-35- (Hart) (Vertical arrows: presenting putative hybrids, the whole names of the samples are given in Table 1).

The second cluster comprised *Q. infectoria* subsps. *infectoria* and subsp. *boissieri* individuals and the two putative hybrids *Q. infectoria* subsp. *infectoria* × *Q. petraea* subsp. *iberica*, and *Q. infectoria* subsp. *infectoria* × *Q. pubescens.* Cluster III contained *Q. virgiliana* and *Q. pubescens* individuals, their putative hybrids *Q. virgiliana* × *Q. pubescens*, *Q. macranthere* subsp. *syspirensis*, and another putative hybrid, *Q. macrenthera* subsp. *syspirensis* × *Q. pubescens*. Finally, cluster IV covered *Q. robur* and *Q. hartwissiana* individuals. *Q. vulcanica*, *Q. pontica* and *Q. petraea* subsp. *petraea* connected to these clusters externally.

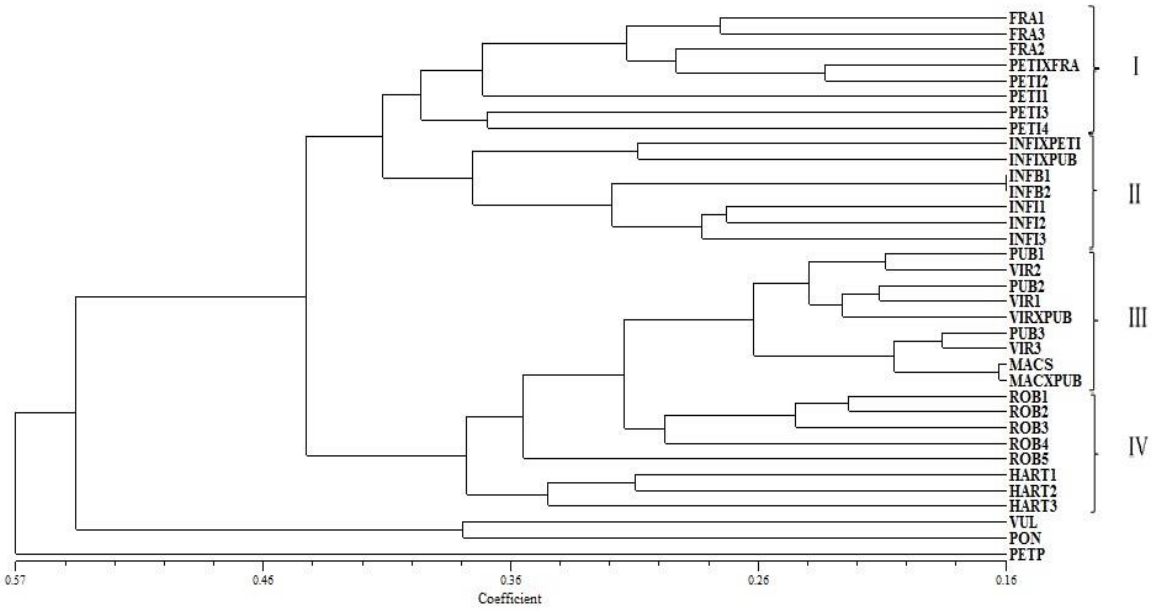


**Figure 3.** Tree diagram (UPGMA) presents genetic relations of white oak individuals and their five putative hybrids. (Roman numbers represent clusters)

In order to reveal genetically similar white taxa more clearly, principal component analysis (PCA) was performed (Figure 4). PCA analysis indicated 46.38% of the total variance (the first axis was 36.54% and the second axis was 9.84%). The PCA diagram presents four main groups with almost similar oaks grouping with the UPGMA dendrogram (Figure 4)

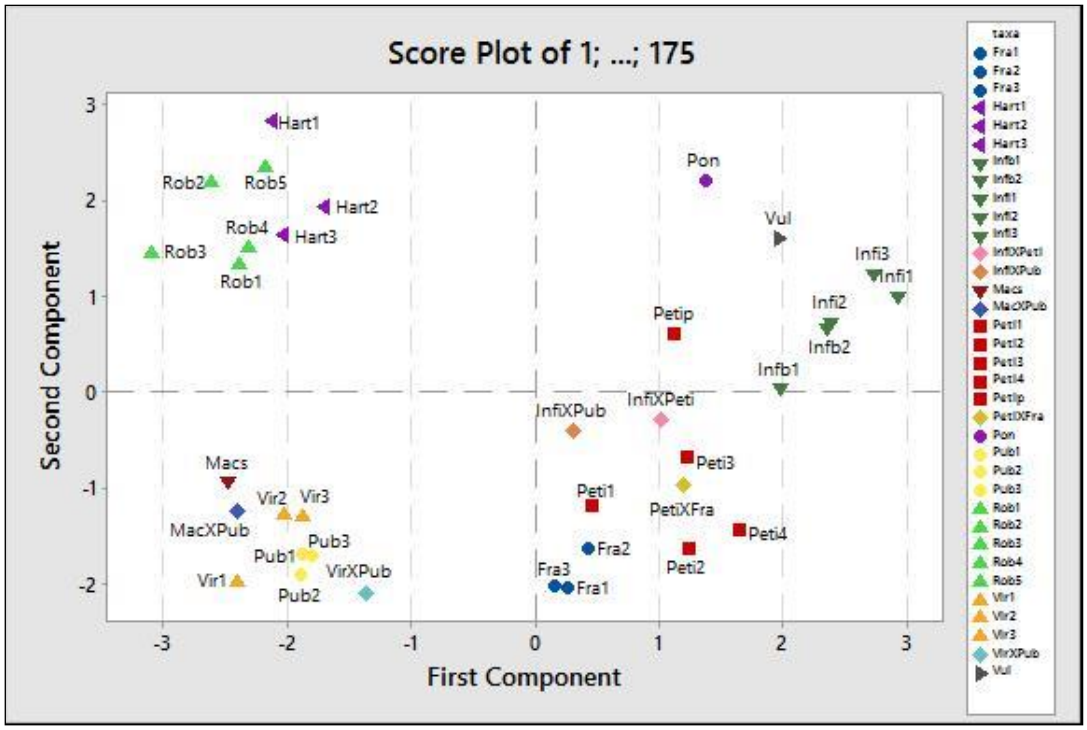


**Figure 4.** PCA diagram, shows the grouping of studied white oak species and their putative hybrids.

The selected ISSR primers showed high polymorphic patterns with *Quercus*. Many studies have demonstrated that the ISSR technique provides high polymorphic fragments and is also useful in differentiating among Fagaceae family individuals (Chokchaichamnanki et al., 2008; Coutinho et al., 2014). ISSR markers were used to identify putative hybrid individuals and relationships between white oak species and some Triticeae members (Carvalho et al., 2005; 2009; Coutinho et al., 2014). Therefore, this research was performed to show genetic relations of studied white taxa including their 5 putative hybrids.

According to the Nei's genetic distance table, the highest genetic distance (0.99) was found between *Q. petraea* subsp *iberica* and *Q. pontica*, and the least distance (0.13) was observed between *Q. robur* taxa (Rob3 and Rob2) (Table 3). Therefore, *Q. pontica* was the most distance species among all the studied *Quercus* taxa. Its geographical distribution is quite limited. It is found in the Caucasus and northeastern Turkey. It grows in regions up to 2000 m such as Trabzon, Artvin, Rize; where the relative humidity is very high (Yaltırık, 1984). This could be the main reason for high genetic distance value from the other white *Quercus* taxa. The second most distance (0.91) taxon was found again between *Q. pontica* and *Q. frainetto* (Table 3).

**Table 3.** Nei's genetic distance matrix of studied *Quercus* taxa

| | FRA1 | FRA2 | FRA3 | PETIXFRA | PETI1 | PETI2 | PETI3 | PETI4 | PETP5 | INFIXPETI | INFB1 | INFB2 | INFI3 | INFI4 | INFI5 | VUL | PON |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| FRA1 | | | | | | | | | | | | | | | | | |
| FRA2 | 0.32 | | | | | | | | | | | | | | | | |
| FRA3 | 0.35 | 0.32 | | | | | | | | | | | | | | | |
| PETIXFRA | 0.34 | 0.29 | 0.41 | | | | | | | | | | | | | | |
| PETI1 | 0.43 | 0.38 | 0.48 | 0.40 | | | | | | | | | | | | | |
| PETI2 | 0.35 | 0.36 | 0.43 | 0.25 | 0.36 | | | | | | | | | | | | |
| PETI3 | 0.43 | 0.48 | 0.45 | 0.44 | 0.41 | 0.36 | | | | | | | | | | | |
| PETI4 | 0.45 | 0.43 | 0.44 | 0.36 | 0.45 | 0.41 | 0.37 | | | | | | | | | | |
| PETP5 | 0.68 | 0.67 | 0.68 | 0.54 | 0.50 | 0.48 | 0.48 | 0.54 | | | | | | | | | |
| INFIXPETI | 0.50 | 0.53 | 0.55 | 0.43 | 0.47 | 0.43 | 0.38 | 0.44 | 0.55 | | | | | | | | |
| INFB1 | 0.47 | 0.41 | 0.48 | 0.37 | 0.55 | 0.42 | 0.51 | 0.37 | 0.68 | 0.42 | | | | | | | |
| INFB2 | 0.58 | 0.47 | 0.56 | 0.40 | 0.57 | 0.49 | 0.46 | 0.42 | 0.61 | 0.41 | 0.18 | | | | | | |
| INFI3 | 0.60 | 0.54 | 0.66 | 0.44 | 0.64 | 0.50 | 0.52 | 0.51 | 0.63 | 0.57 | 0.31 | 0.24 | | | | | |
| INFI4 | 0.56 | 0.49 | 0.54 | 0.51 | 0.45 | 0.45 | 0.41 | 0.49 | 0.54 | 0.39 | 0.35 | 0.27 | 0.37 | | | | |
| INFI5 | 0.58 | 0.48 | 0.54 | 0.48 | 0.43 | 0.50 | 0.52 | 0.53 | 0.63 | 0.46 | 0.38 | 0.25 | 0.30 | 0.28 | | | |
| VUL | 0.80 | 0.83 | 0.80 | 0.63 | 0.86 | 0.83 | 0.76 | 0.86 | 0.86 | 0.61 | 0.57 | 0.61 | 0.65 | 0.66 | 0.59 | | |
| PON | 0.75 | 0.85 | 0.91 | 0.69 | **0.99** | 0.88 | 0.72 | 0.85 | 0.88 | 0.72 | 0.68 | 0.62 | 0.60 | 0.55 | 0.54 | 0.53 | |
| PUB1 | 0.33 | 0.42 | 0.52 | 0.38 | 0.56 | 0.46 | 0.55 | 0.45 | 0.69 | 0.63 | 0.55 | 0.62 | 0.69 | 0.73 | 0.67 | 0.68 | 0.73 |
| PUB2 | 0.45 | 0.47 | 0.49 | 0.45 | 0.55 | 0.58 | 0.57 | 0.56 | 0.58 | 0.53 | 0.54 | 0.58 | 0.74 | 0.79 | 0.56 | 0.77 | 0.91 |
| PUB3 | 0.51 | 0.48 | 0.49 | 0.42 | 0.50 | 0.49 | 0.54 | 0.53 | 0.63 | 0.52 | 0.61 | 0.63 | 0.77 | 0.69 | 0.63 | 0.77 | 0.84 |
| VIRXPUB | 0.57 | 0.49 | 0.53 | 0.51 | 0.55 | 0.57 | 0.51 | 0.50 | 0.74 | 0.51 | 0.55 | 0.65 | 0.75 | 0.70 | 0.63 | 0.69 | 0.84 |
| INFIXPUB | 0.43 | 0.34 | 0.38 | 0.37 | 0.41 | 0.38 | 0.41 | 0.46 | 0.48 | 0.38 | 0.34 | 0.34 | 0.45 | 0.42 | 0.41 | 0.68 | 0.76 |
| VIR1 | 0.48 | 0.50 | 0.53 | 0.52 | 0.62 | 0.60 | 0.61 | 0.58 | 0.82 | 0.54 | 0.65 | 0.72 | 0.86 | 0.88 | 0.70 | 0.88 | 0.83 |
| VIR2 | 0.45 | 0.43 | 0.56 | 0.49 | 0.57 | 0.52 | 0.61 | 0.61 | 0.73 | 0.51 | 0.63 | 0.65 | 0.71 | 0.58 | 0.55 | 0.85 | 0.69 |
| VIR3 | 0.47 | 0.46 | 0.47 | 0.49 | 0.53 | 0.54 | 0.50 | 0.57 | 0.67 | 0.58 | 0.54 | 0.69 | 0.79 | 0.60 | 0.70 | 0.81 | 0.72 |
| MACS | 0.48 | 0.50 | 0.42 | 0.53 | 0.60 | 0.55 | 0.59 | 0.67 | 0.71 | 0.57 | 0.58 | 0.60 | 0.77 | 0.76 | 0.68 | 0.77 | 0.88 |
| MACSXPUB | 0.47 | 0.49 | 0.45 | 0.49 | 0.53 | 0.54 | 0.54 | 0.67 | 0.68 | 0.60 | 0.59 | 0.68 | 0.71 | 0.64 | 0.63 | 0.80 | 0.79 |
| ROBR1 | 0.53 | 0.51 | 0.53 | 0.42 | 0.71 | 0.53 | 0.56 | 0.55 | 0.79 | 0.52 | 0.54 | 0.58 | 0.68 | 0.63 | 0.58 | 0.69 | 0.68 |
| ROBR2 | 0.53 | 0.50 | 0.57 | 0.52 | 0.64 | 0.53 | 0.57 | 0.64 | 0.80 | 0.52 | 0.54 | 0.60 | 0.61 | 0.61 | 0.55 | 0.71 | 0.63 |
| ROBR3 | 0.43 | 0.42 | 0.45 | 0.44 | 0.58 | 0.48 | 0.52 | 0.57 | 0.66 | 0.46 | 0.49 | 0.57 | 0.66 | 0.54 | 0.59 | 0.67 | 0.68 |
| ROBR4 | 0.45 | 0.47 | 0.52 | 0.37 | 0.51 | 0.45 | 0.57 | 0.65 | 0.72 | 0.53 | 0.54 | 0.59 | 0.70 | 0.62 | 0.56 | 0.83 | 0.78 |
| ROBR5 | 0.54 | 0.42 | 0.52 | 0.42 | 0.56 | 0.48 | 0.65 | 0.70 | 0.72 | 0.58 | 0.57 | 0.62 | 0.67 | 0.62 | 0.59 | 0.81 | 0.88 |
| HART1 | 0.59 | 0.57 | 0.57 | 0.49 | 0.62 | 0.61 | 0.67 | 0.66 | 0.66 | 0.66 | 0.64 | 0.59 | 0.73 | 0.69 | 0.66 | 0.77 | 0.69 |
| HART2 | 0.62 | 0.48 | 0.51 | 0.48 | 0.57 | 0.60 | 0.56 | 0.48 | 0.67 | 0.52 | 0.54 | 0.60 | 0.70 | 0.63 | 0.62 | 0.78 | 0.84 |
| HART3 | 0.47 | 0.44 | 0.52 | 0.42 | 0.53 | 0.56 | 0.57 | 0.61 | 0.61 | 0.60 | 0.59 | 0.59 | 0.71 | 0.66 | 0.56 | 0.85 | 0.70 |

**Table 3.** Nei's genetic distance matrix of studied *Quercus* taxa (continue)

| | PUB1 | PUB2 | PUB3 | VIRXPUB | INFIXPUB | VIR1 | VIR2 | VIR3 | MACS | MACSXPUB | ROBR1 | ROBR2 | ROBR3 | ROBR4 | ROBR5 | HART1 | HART2 | HART3 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| FRA1 | | | | | | | | | | | | | | | | | | |
| FRA2 | | | | | | | | | | | | | | | | | | |
| FRA3 | | | | | | | | | | | | | | | | | | |
| PETIXFRA | | | | | | | | | | | | | | | | | | |
| PETI1 | | | | | | | | | | | | | | | | | | |
| PETI2 | | | | | | | | | | | | | | | | | | |
| PETI3 | | | | | | | | | | | | | | | | | | |
| PETI4 | | | | | | | | | | | | | | | | | | |
| PETP5 | | | | | | | | | | | | | | | | | | |
| INFIXPETI | | | | | | | | | | | | | | | | | | |
| INFB1 | | | | | | | | | | | | | | | | | | |
| INFB2 | | | | | | | | | | | | | | | | | | |
| INFI3 | | | | | | | | | | | | | | | | | | |
| INFI4 | | | | | | | | | | | | | | | | | | |
| INFI5 | | | | | | | | | | | | | | | | | | |
| VUL | | | | | | | | | | | | | | | | | | |
| PON | | | | | | | | | | | | | | | | | | |
| PUB1 | | | | | | | | | | | | | | | | | | |
| PUB2 | 0.23 | | | | | | | | | | | | | | | | | |
| PUB3 | 0.20 | 0.19 | | | | | | | | | | | | | | | | |
| VIRXPUB | 0.29 | 0.22 | 0.26 | | | | | | | | | | | | | | | |
| INFIXPUB | 0.37 | 0.31 | 0.35 | 0.35 | | | | | | | | | | | | | | |
| VIR1 | 0.31 | 0.28 | 0.27 | 0.29 | 0.49 | | | | | | | | | | | | | |
| VIR2 | 0.33 | 0.26 | 0.27 | 0.32 | 0.38 | 0.28 | | | | | | | | | | | | |
| VIR3 | 0.38 | 0.40 | 0.28 | 0.33 | 0.37 | 0.36 | 0.25 | | | | | | | | | | | |
| MACS | 0.42 | 0.35 | 0.34 | 0.35 | 0.41 | 0.36 | 0.38 | 0.37 | | | | | | | | | | |
| MACSXPUB | 0.34 | 0.32 | 0.25 | 0.38 | 0.38 | 0.34 | 0.30 | 0.25 | 0.21 | | | | | | | | | |
| ROBR1 | 0.36 | 0.32 | 0.32 | 0.42 | 0.43 | 0.38 | 0.35 | 0.31 | 0.39 | 0.33 | | | | | | | | |
| ROBR2 | 0.38 | 0.34 | 0.34 | 0.35 | 0.45 | 0.35 | 0.34 | 0.35 | 0.41 | 0.35 | 0.22 | | | | | | | |
| ROBR3 | 0.28 | 0.25 | 0.26 | 0.32 | 0.37 | 0.31 | 0.26 | 0.30 | 0.30 | 0.25 | 0.17 | **0.13** | | | | | | |
| ROBR4 | 0.36 | 0.33 | 0.32 | 0.38 | 0.38 | 0.36 | 0.33 | 0.35 | 0.37 | 0.29 | 0.29 | 0.28 | 0.20 | | | | | |
| ROBR5 | 0.42 | 0.39 | 0.40 | 0.43 | 0.41 | 0.41 | 0.37 | 0.35 | 0.40 | 0.37 | 0.32 | 0.29 | 0.22 | 0.18 | | | | |
| HART1 | 0.42 | 0.42 | 0.45 | 0.60 | 0.50 | 0.46 | 0.41 | 0.42 | 0.49 | 0.46 | 0.41 | 0.47 | 0.39 | 0.35 | 0.36 | | | |
| HART2 | 0.47 | 0.48 | 0.43 | 0.39 | 0.42 | 0.42 | 0.37 | 0.37 | 0.43 | 0.44 | 0.39 | 0.43 | 0.32 | 0.33 | 0.40 | 0.28 | | |
| HART3 | 0.39 | 0.36 | 0.36 | 0.50 | 0.36 | 0.41 | 0.33 | 0.37 | 0.44 | 0.39 | 0.36 | 0.42 | 0.32 | 0.26 | 0.31 | 0.30 | 0.30 | |

Similarly, *Q. pontica* and *Q. vulcanica* were not included in any of the main groups in UPGMA dendrogram (Figure 3), but they were placed partly close to the group 2 taxa (*Q. infectoria* individuals) in PCA results. The results of UPGMA separated mainly four groups, which were also supported by PCA results (Figures 3-4). These ungrouped taxa presented single samples from geographically distant regions, and they did not show much genetic similarities with others. Consequently, individuals of the

same taxa were grouped together in both results. In addition, it came into view that the projections of the hybrid groups were located usually between their potential parental individuals (Figure 4).

In the literature, these white oak species were also found close to each other. For instance, morphological variation in mixed oak areas consists of *Q. robur* and *Q. petraea* which are widely scattered species in Europe (Kremer et al., 2002). *Q. petraea*, *Q. pubescens* and *Q. robur* are usually found closely related (Finkeldey & Mátyás, 2003). However, *Q. robur*, *Q. petraea* and *Q. pubescens* species did not show any established molecular affinity in our tree dendrogram. On the other hand, there are other studies, which have found similar results with our study such as, in the research of ITS in Italian oaks, *Q. petraea*, *Q. frainetto* and *Q. robur* were found in the same cluster (Bellarosa et al., 2005). In another study, hybrid occurred between *Q. robur*, *Q. petraea* and *Q. frainetto* (Curtu et al., 2007*a, b*). A high gene flow was identified among *Q. petraea*, *Q. frainetto* and *Q. pubescens* in the research conducted by Italian oaks (Antonecchia et al., 2015). According to their research, hybridization mostly occurred between *Q. petraea* and *Q. pubescens* while hybrids between *Q. frainetto* and *Q. petraea* were observed rarely. Taxonomically, controversial species of *Q. virgiliana* was found as a putative hybrid between *Q. petraea* and *Q. pubescens* in a morphological study (Borazan & Babaç, 2003). However, *Q. virgiliana* as an intraspecific taxonomic unit differs from *Q. pubescens* species (Enescu et al., 2013). A putative hybrid of *Q. virgiliana* and *Q. pubescens* was detected in this study, and these two taxa were found very close to each other (Figure 3). Another study with SSR markers showed high affinity between *Q. pubescens* and *Q. virgiliana* (Fortini et al., 2009) which supports the results of this study. Furthermore, two other putative hybrids between *Q. infectoria*, *Q. pubescens* and *Q. petraea* were also reported in this study. *Q. infectoria* is quite common in Turkey (Uslu et al., 2011) and might have hybrids between them (Kasaplıgil, 1992). The last hybrid between *Q. macrenthera* subsp. *syspirensis* and *Q. pubescens* was also recognized in this study. This hybrid occurs in mixed forest formation with *Quercus pubescens* (Hedge & Yaltırık, 1982). Thus, production of hybrid between these species is highly possible according to their geographical distributions (Uslu et al., 2011).

**Conclusions**

Genetic relations of studied white oaks were presented in this study. Genetically the most distance was found between Q. pontica and Q. petraea. Secondly, Q. pontica and Q. frainetto. Ecological conditions and geographical difference of Q. pontica may have caused this relation. On the other hand, the same taxon individuals such as Rob3 and Rob4 were found to be the closest taxa.

The formation of hybrids is a very natural result because the external pollination is quite common among the oak taxa. To identify these hybrids are not an easy job by morphological methods. In this study, molecular markers (ISSR) were used to contribute to the solution of this problem. Three of the five putative hybrids (*Q. infectoria* subsp. *infectoria* × *Q pubescens*, *Q. virgiliana* × *Q. pubescens* and *Q. petraea* subsp. × *Q. frainetto*) were demonstrated distinctly by ISSR makers. This is also clearly

shown in groupings in PCA analyzes. Finally, the results of this study confirmed that ISSR markers produce high polymorphism among the Turkish white oak species and might be useful in differentiating possible hybrids in oaks and related species.

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**Conflict of Interest**

The authors affirm that they have no conflict of interest.

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