PATTERN OF GENETIC DIVERSITY IN TURKEY OAK (*QUERCUS CERRIS* L.) POPULATIONS

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ABSTRACT

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Quercus cerris L. is a native and widely distributed species in Turkey. It is an important element of Anatolian forests and with other oak species constitute 76.4% of broadleafed forests. However, there is little information on the genetic make-up of Turkish populations. In the current study, the genetic diversity of *Q. cerris* populations sampled within their natural range is assessed with microsatellite loci (SSRs) polymorphism.

Thirteen populations were sampled from seven different locations. Four of these are rich in different oak species coexisting and possibly hybridizing. To estimate population genetic diversity parameters, twelve microsatellite primers were applied to 172 *Q. cerris* genotypes and 47 suspected hybrid genotypes. All genetic analysis were carried out in duplicate. Also, in order to evaluate the genetic proximity

between *Q. cerris* and other oaks with suspected hybrid genotypes, Principle Component Analysis (PCA) were conducted for different regions.

It was found that the highest molecular diversity was detected in Kahramanmaraş-Adıyaman region's populations. The populations that had the least genetical diversity were from Kastamonu and Ankara. According to the UPGMA dendrogram results, two major clusters were defined. One of them was composed of Kastamonu-Ankara populations while the other cluster had the rest of the studied populations. When the most probable number of populations (*K*) was estimated, it was found to be two for just *Q. cerris* genotypes and nine for *Q. cerris* genotypes analyzed with 47 suspected hybrid genotypes. For nine clusters, Kahramanmaraş-Adıyaman populations and Kastamonu-Ankara populations formed big clusters as the rest formed their individual clusters. Among suspected hybrid genotypes, except six of them, all was inferred to have certain type of populational genomic composition with a minimum 0.93 membership of coefficient for these populations. Among six, four of them had coefficients less than 0.80 and membership of two or three populations.

As a result of this study, pattern of genetic diversity in Turkey oak obtained for the first time. This genetic information will be useful for establishing principles for conservation and management of genetic resources of the species since most of Turkey oak dispersion ranges are faced with a serious degradation rates.

Key words: *Quercus cerris* L., genetic diversity, microsatellites, hybridization

ÖZ

TÜRK MEŞESİ (*QUERCUS CERRIS* L.) POPULASYONLARINDA GENETİK ÇEŞİTLİLİĞİN YAPILAŞMASI

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Quercus cerris L., Türkiye'de doğal olarak yetişen ve geniş yayılım gösteren bir türdür. Anadolu ormanlarının önemli bir bileşenidir ve diğer meşe türleriyle birlikte geniş yapraklı ormanların %76,4'ünü oluşturmaktadır. Ancak Türk populasyonlarının genetik yapısı hakkında çok az miktarda bilgi mevcuttur. Bu çalışmada, doğal yayılım alanlarından örneklenmiş *Q. cerris* populasyonlarının genetik çeşitliliği, mikrosatellit lokusları (SSRs) polimorfizmiyle değerlendirilmektedir.

Yedi farklı bölgeden on üç populasyon örneklenmiştir. Bunlardan dört tanesi, beraberce yayılım gösteren ve muhtemelen melezleşen farklı meşe türleri açısından zengindir. Populasyon genetik çeşitlilik parametrelerini hesaplamak için on iki mikrosatellit işaretleyicisi 172 *Q. cerris* genotipine ve 47 ekstra olası melez bireyine uygulanmıştır. Tüm genetik analizler ikişer kere yapılmıştır. Ayrıca, *Q. cerris* ve

diğer meşe türleri ile olası melez bireylerinin genetik yakınlığını değerlendirebilmek için farklı bölgeler için Temel Bileşen Analizi (PCA) yapılmıştır.

En yüksek moleküler çeşitlilik Kahramanmaraş-Adıyaman bölgesinde tespit edilmiştir. En düşük genetik çeşitliliğe sahip populasyonlar ise Kastamonu ve Ankara populasyonlarıdır. UPGMA kümelendirme sonuçlarına göre iki temel küme tanımlanmıştır. Bunlardan biri Kastamonu-Ankara populasyonlarından oluşurken, diğer küme, kalan tüm populasyonları içermektedir. En olası gerçek populasyon sayısı (*K*) tahmin edildiğinde, sadece *Q. cerris* genotipleri için iki, *Q. cerris* genotipleri ile 47 olası melez bireyleri için ise dokuz bulunmuştur. Dokuz küme için, Kahramanmaraş-Adıyaman populasyonları ve Kastamonu-Ankara populasyonları büyük kümeler oluştururken, kalan tüm populasyonlar kendi kümelerini oluşturmuştur. Olası melez bireyleri arasında, altı tanesi hariç, tümünün minimum 0.93 aitlik katsayısı ile belli bir tip genomik kompozisyona sahip olduğu sonucuna ulaşılmıştır. Altısı içinden dördü de 0.80 aitlik katsayısına sahiptir ve iki ya da üç populasyona üyelikleri tespit edilmiştir.

Bu çalışmanın bir sonucu olarak, Türk meşesinin genetik çeşitliliğinin yapılaşması ilk kez elde edilmiştir. Bu genetik bilgi, özellikle Türk meşesi yayılım alanlarının ciddi bir tahbibatla karşı karşıya olduğu düşünüldüğünde, türün genetik kaynaklarının korunması ve idaresi amacıyla ilkeler oluşturmak açısından yararlı olacaktır.

Anahtar kelimeler: *Quercus cerris* L., genetik çeşitlilik, mikrosatellitler, melezleşme

Dedicated to my parents

Serpil TÜMBİLEN and Birol TÜMBİLEN

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In the memory of my dear older sister Yeşim TÜMBİLEN, who will always be remembered and loved.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

cDNA	Complementary DNA
cpDNA	Chloroplast DNA
CLUMPP	Cluster Matching and Permutation Program
CTAB	Cetyl trimethyl ammonium bromide
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide triphosphate
EDTA	Ethylenediaminetetraaceticacid disodium salt
EST	Expressed sequence tags
GDA	Genetic Data Analysis
ISSR	Inter-simple sequence repeats
ITS	Internal transcribed spacer
MCMC	Markov chain Monte Carlo
NJ	Neighbour-joining
NTSYS-Pc	Numerical taxonomy and multivariate analysis system
PCA	Principle component analysis
PCR	Polymerase chain reaction
PVP	Polyvinylpyrrolidone
RFLP	Restriction fragment lenght polymorphism
RPM	Revolutions per minute
SSR/STR	Simple sequence repeats / Simple tamdem repeats
TBE	Tris-Borate-EDTA
TE	Tris EDTA
UPGMA	Unweighted pair group method with arithmetic mean
VNTR	Variable number tandem repeats

CHAPTER 1

INTRODUCTION

Turkey is a Eurasian country consisting of a large Anatolian peninsula and a smaller European part. It is situated in between Asia and Europe and neighbours to Middle East, Caucasus and Balkans. It is located in temperate zone where different climate types are experienced conspicuously. Turkey has a high average altitude which gradually rises from west to east. Along with its different land forms and soil types, fluctuations in elevation in short ranges, climatic situation and marine influences result in great biodiversity and high levels of endemism in Turkey.

In the past, botanists and biogeographists have tried to categorize geographic areas on earth having certain types of botanical richness and endemism. These geographic areas are called as phytochoria in common and phytogeographical (floristic) kingdoms, regions, provinces are some hierarchical units of this terminology (Avcı, 1996). According to Drude, floral regions are the units which exhibit high degree of generic endemism (Avcı, 1996). Humboldt, Grisebach, Good, Drude, Zohary and Davis are some of the important researchers in this field (Avcı, 1996). In Turkey, two scientists' views have became more of an issue: Davis and Zohary (Davis, 1965; Davis, 1971; Zohary, 1971; Zohary, 1973; Avcı, 1996). The main difference in their points of view was the interpretation of past records while defining floristic groups. As Zohary taking historical records of vegetation into account (Zohary, 1971), Davis appreciates todays' vegetation cover of regions (Davis, 1971; Avcı, 1996).

Fundamentally, 3 types of phytogeographical regions are defined for Turkey that are the Euro-Siberian, Mediterranean and Irano-Turanian (Davis, 1971; Zohary, 1971).

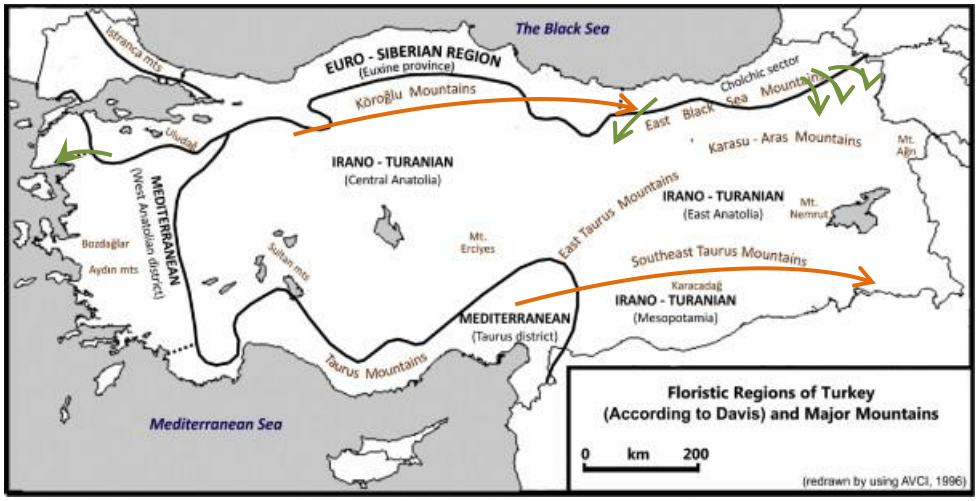


Figure 1 A redrawn version of floristic regions of Turkey
(Orange arrows: The Mediterranean penetrations;
Green arrows: The Euro-Siberian penetrations)
(Köse et al., 2012)

The Euro-Siberian region covers the entire Black Sea coast and the Black Sea coast of Thrace. Large leaved deciduous forests, shrubs at lower altitudes where the original plant cover has been destroyed, and conifers at higher altitudes are the characteristic flora of this region (Çolak and Rotherham, 2006). A rainy climate throughout the year with high precipication ratio and low seasonal temperature differences and foggy wheather are climatic conditions (Çolak and Rotherham, 2006). The Mediterranean region includes western and southern part of the Anatolia and the Amanos mountains. Maquis and dry forests at high elevations and dry, hot summers with warm and rainy winters are the characteristics of the region. The remaining inner, eastern and southeastern Anatolia are defined as the Irano-Turanian floristic region. In this region, winters are cold and snowy, summers are usually hot and dry. Daily temperature differences are high, and the rainfall amount is low. Plant cover that is adapted to these climatic conditions is predominantly steppe vegetation and dry forests.

Each of these regions mentioned above has specific vegetational and climatic conditions. However, there are no sharp interruption of regional features that there are transition areas where regional influences penetrates into others and evantuate a zone sheltering both plant cover with high endemic ratio and special vegetation types (Davis, 1971; Avcı, 1996). These are the extensions of the Mediterranean region to the Irano-Turanian, Colchic sector to the Irano-Turannian, the Mediterranean penetration to the Irano-Turanian upon the Taurus mountains and the Euro-Siberian extension to the Irano-Turanian through the mid-Black Sea Region. These penetrations which were originally defined by Davis (1971) indicated by colored arrows in Figure 1.

Forest ecosystems are the major ecosystem types of Turkey (Kaya and Raynal, 2001). As a consequence of the factors affecting plant diversity in Turkey described above, there are different types of forests with more than 450 tree species and shrubs (Çolak and Rotherham, 2006). According to the Forestry Statistics of Ministry of Forestry and Water Affairs (2010), which are renewed in every five years, forests are classified into two groups as high or coppice forests. These forests covers a total 21.5 million ha (Forestry Statistics, 2010). Of this amount, 77% constitue high forests and the remaining 23% represent coppice forests (Forestry Statistics, 2010). When the forests of Turkey are examined according to forest types, 61% forest lands are coniferous and 39% are broadleaved (Forestry Statistics, 2010). Detailed information on forests in Turkey were given in Table 1 and Table 2 (Forestry Statistics, 2010).

Table 1 High and Coppice Forests' dispersion ranges

	Total	**Productive**	**Degraded**
High forest	16,662,379	9,782,513	6,879,866
Coppice forest	4,874,712	1,420,324	3,454,388
Total	**21,537,091 ha**	**11,202,837 ha**	**10,334,254 ha**

Table 2 Turkey forests' forms and their coverage amounts on the country

Forest form	Total	Coniferous	Broadleafed
Productive	11,202,837	7,395,640	3,807,197
Degraded	10,334,254	5,763,134	4,571,120
Total	**21,537,091 ha**	**13,158,774 ha**	**8,378,317 ha**

Fagaceae family members in Turkey (chesnut, beech and oak) account for more than 8 million ha which is close to the whole decidious forests' area (Forest Atlas, 2012). Among them, oaks have pivotal importance. Total of 6.4 million ha of Turkey, constituting 76.4 % of broadleafed forests is covered by oak species (Çolak and Rotherham, 2006; Forest Atlas, 2012). This makes the genus have the highest distribution area and percentage among broadleafed tree species.

1.1 Fagaceae Family: Genus *Quercus* L.

The Fagaceae or beech family is a member of flowering plants and one of the families of Fagales order. Some of the best known tree species such as chesnut, beech and oak are the members of the family. It includes nine genera and about 900 species (Judd et al., 2002). Family members are trees or shrubs and have a good dispersal in tropical, temperate and seasonally dry regions of the Northern hemisphere (Manos et al., 2001; Judd et al., 2002). The familial ancestral origin is stated as the Southeast Asia and Pasific Islands (Kaul, 1985; Manos et al., 2001). Major characteristics are unisexual flowers, nutty fruits without endosperm and often

lobed leaves with stipules (Judd et al., 2002). Different pollination vectors are observed for the family members such as wind, insects and mammals although wind pollination is a general feature of Fagales (Manos et al., 2001; Judd et al., 2002). There are economically important taxa, members of which used in construction works, some others have edible nuts and few of as ornamentals (Judd et al., 2002).

Among nine Fagaceae genera, the genus *Quercus* is the best known and the largest genus of the family, including around 450 species (Kaul, 1985; Manos et al., 2001; Judd et al., 2002). Members of the genus are decidious or evergreen forests and shrubs which are anemophilous (Kaul,1985; Nixon, 1993). Species are monoecious and female flowers are as spikes and males forms catkins (Kaul,1985; Nixon 1993; Judd et al., 2002). Fruits are nuts in cupules of which formation is an important indicator in phylogeny (Manos et al., 2001) and they mature in 1-2 years (Kaul, 1985; Nixon 1993). In general, species are diploid and have a haploid chromosome number 12 (D'emerico et al., 1995; Yılmaz et al., 2011). Besides, though it is rare, polyploid species were observed in certain studies (Johnsson, 1946; Burda, 1973; Butorina, 1993; Dzialuk, 2007).

With respect to paleontologic investigations, the history of abundance of oaks were found to date back to 40 million years to Cenozoic Era (Nixon 1993; Manos et al., 1999). Today, they spread out to temperate and dry forests of America, Europe and Asia in the Northern hemisphere (Kaul, 1985; Manos et al., 1999). The center of diversity of the genus *Quercus* is Central and Eastern Asia (Kaul, 1985) although with higher number of species present in Central America and Southern United States (Kaul, 1985; Nixon, 1993; Manos et al., 2001).

There are several diseases and pests affect oak species, most of which are caused by fungi. Sudden oak death and oak wilt are important ones as there are also powdery mildew, gall formations on several organs of oak trees, wood-boring beetles etc. Fortunately, white oak members due to wood anatomy, abundant tyloses in the heartwood, are invulnerable to diseases than red oaks (Nixon, 2007). Therefore, they are also more preferrable in barrel production and flooring (Nixon, 2007).

Hybrid formation is a common situation which makes the taxonomy of the genus complicated. It is such a situation that Whittemore and Schaal (1991) questioned traditional species concept and emphasized the genus *Quercus* is beyond the limits (Rushton, 1993; Curtu et al., 2007). Most of the investigations about hybrids and the genus have been upon morphological data especially leaf and fruit. However, what makes just morphology is not enough to define hybrids in *Quercus* is due to great morphological differences already exist in possible parental species (Rushton, 1993). Environmental conditions may lead to variation in leaf and fruit morphology even on the same tree (Davis, 1971; Rushton, 1993). For this reason, additional properties should be taken into consideration for a more reliable comment of classification onthe genus (Gottlieb, 1972). For this aim, molecular data has been incorporated into the systematics of *Quercus*. Another reason of the complexity of classification is because of varied names for different groups (Nixon, 2007) and significant changes in groupings as a result of studies done over time (Nixon,1993; Manos et al., 1999; Manos et al., 2001).

Basically, genus *Quercus* divided into two subgenera which are *Quercus* and *Cyclobalanopsis* (Kaul, 1985; Nixon, 1993; Manos et al., 1999; Judd et al., 2002). As *Cyclobalanopsis* has rather confined to Southeastern Asia and Malaysia and has less species, subgenus *Quercus* possesses most of the species with a broader dispersal area (Kaul, 1985; Nixon, 1993; Manos et al., 2001; Nixon, 2007). There are 76 *Cyclobalanopsis* species and 320-354 *Quercus* species and most of the *Quercus* subgenus members reside in America (https://w3.pierroton.inra.fr/QuercusPortal/). It was noted that Europe has 22 and Asia has 98 species of subgenus (https://w3.pierroton.inra.fr/QuercusPortal/). In Figure 2, the dispersion of two subgenera of the genus *Quercus* was given.

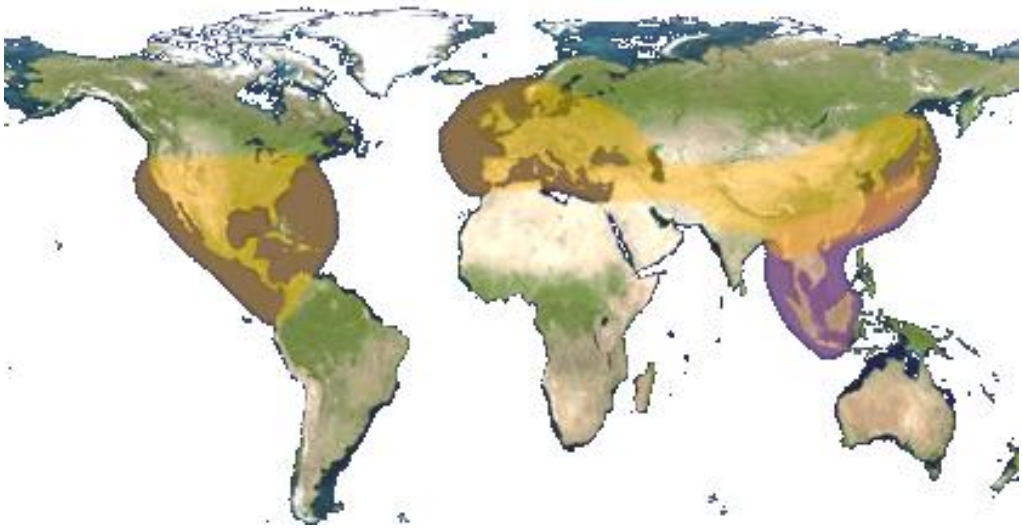


Figure 2 Dispersion of *Quercus* species on Earth
(Yellow area: subgenus *Quercus*; Pink area: subgenus *Cyclobalanopsis*)
(https://w3.pierroton.inra.fr/QuercusPortal/index.php?p=BIODIVERSITY)

Subgenus *Quercus* is the more problematic taxa of the genus. Over time, sectional arrangements of the subgenus has changed as new data emerged. In general sense, three sections have been defined (Nixon 1993; Manos et al., 1999; Manos et al., 2001; Judd et al., 2002). These are red oaks (*Lobatae*), intermediate oaks (*Protobalanus*) and white oaks (*Quercus- Lepidobalanus*) (Nixon, 1993; Manos et al., 1999; Manos et al., 2001; Judd et al., 2002). Biennial or annual fruit maturation, either bristle tipped or non-bristle tipped leaves, abortion of ovules near apex or at the base of the nut are the features of red and white oaks respectively (Judd et al., 2002). For the intermediate oaks, fruits can mature either annually or biennially and fruit wall structure may be glabrous or pubescent (Judd et al., 2002). Their dispersal are also different. Red oaks and intermediate oaks have species residing in the New World while white oaks have a wider range and found both in the New World and in the Old World covering North and Central America and Eurasia (Nixon, 1993). *Cerris* and *Ilex* have been defined as groups in the subgenus *Quercus* section *Quercus* according to morphological data (Manos et al., 1999). The most recent morphological phylogenetic classification of genus *Quercus* was given in Figure 3. However, in studies of Manos and colleagues (1999; 2001), the results from DNA

(Deoxyribonucleic acid) analysis of nuclear and extrachromosomal structures indicated that these groups as a seperate section: *Cerris* (Manos et al., 1999; Manos et al., 2001; Nixon, 2007; Burgarella et al., 2009). These findings support Camus's studies (1936-1954) who had previously defined *Cerris* as an Old World section of subgenus *Quercus* (Kaul, 1985).

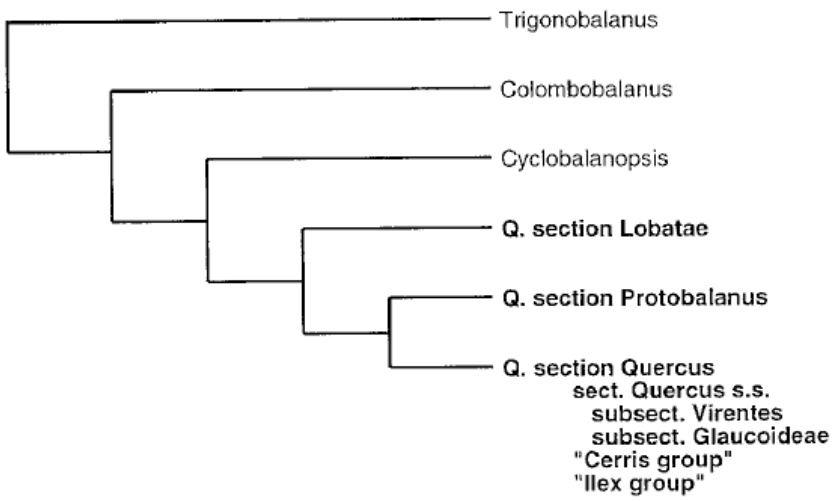


Figure 3 The most recent phylogenetic classification of genus *Quercus* (Manos et. al., 1999)

When Turkey is evaluated in terms of oak species diversity, the genus has 18 species which are classified into three sections; *Ilex*, *Cerris* and *Quercus* (Davis, 1971; Hedge and Yaltırık, 1982). A section is a taxonomic rank between genus and if defined subgenus and species. The sectional arrangement of Turkish oaks in the subgenus *Quercus* is similar to the worldwide classification of taxonomic ranks of the subgenus *Quercus*. These sections are constituted according to anatomical wood structure, fruit maturation times, leaf and bark properties of species (Hedge and Yaltırık, 1982). The list of Turkish oak species is given in Table 3.

Table 3 *Quercus* species naturally grown in Turkey
(http://web.ogm.gov.tr/BilgiServisleri/mese/gb.htm)

Section *Cerris*	**Section *Quercus***		**Section *Ilex***
Q. libani Oliv.	*Q. robur* L.	*Q. pontica* K. Koch	*Q. coccifera* L.
Q. trojana Webb	*Q. petraea* (Matt.) Liebl.	*Q. infectoria* Oliv.	*Q. ilex* L.
Q. cerris L.	*Q. frainetto* Ten.	*Q. pubescens* Willd.	*Q. aucheri* Jaub.&Spach
Q. brantii Lindl.	*Q. vulcanica* Boiss.&Heldr. ex Kotschy	*Q. macranthera* Fisch.&Mey ex Hohen.	
Q. ithaburensis ssp. *macrolepis* (Kotschy) Hedge&Yalt.	*Q. hartwissiana* Steven	*Q. virgiliana* Ten.	

Each section members have special features. The main differences are fruit maturation times, inner acorn shell structure, taste of nuts due to presence of tannins and leaf edge morphology. With regard to them, white oaks of Turkey have one year fruit maturation time, non-bristled tips, acorn shell inside is hairless and low tanin amount makes the nuts edible. Red oaks have two years acorn maturation time, bristle-tipped leaves. The inside of acorn shell is woolly, also the acorns taste bitter. Evergreen oaks members have either red oaks' features or white oaks' features (Hedge and Yaltırık, 1982), but they are characterized with non-deciduous leaves.

Oak species have a well distribution in every region of Turkey and all three phytogeographical regions. As the other broadleafed forest trees, oaks are predominantly occupy lower elevations (Şekercioğlu et al., 2011) and mostly found in the mountains (Çolak and Rotherham, 2006). They can form either pure oak stands or mixed forests with coniferous species (Kaya and Raynal, 2001; Çolak and Rotherham, 2006). A general distribution of oak species in the country is illustrated in Figure 4 (Forestry Statistics, 2010). In Table 4, forest types in Turkey according to floristic and geographic regions specifically where *Quercus spp*. are distributed is given (Rearranged according to Kaya and Raynal, 2001).

Although 6.4 million ha is covered by oaks, more than 65% of this amount is degraded forests (Forest Atlas, 2012). This degradation and decrease in forest areas not only for oak species but also for all forest trees in the country, are substantially a result of antropogenic effect especially in the last five hundred years (Çolak and Rotherham, 2006). Habitat alteration, fragmentation and loss, over-use of woody and non-woody forest constituent, environmental pollution and introduction of alien species are some of the reasons for degradation (Kaya and Raynal, 2001; Çolak and Rotherham, 2006; Şekercioğlu et al., 2011). Fires whether intentionally or unintentionally or due to natural reasons have been an important threat for Turkey's forests (Şekercioğlu et al., 2011). Although 95% of degraded and deforested areas of the country are considered that it is possible to reforest, their opening to settlement or use by goverments results in permanent loss of these areas (Şekercioğlu et al., 2011). Despite these, natural values and biodiversity have been tried to be protected by several laws and regulations which cover protection, maintenance, refinement and

utilization (Kaya and Raynal, 2001). There are also replantation efforts of areas that were originally forested by goverments and civil initiatives and organizations (Şekercioğlu et al., 2011). Also, fortunatelly Anatolian forests' are defined as having their naturallness in general and have a large potential of genetic diversity (Çolak and Rotherham, 2006). *Q. cerris* is a native member of Turkey's forests and should be taken into consideration for genetic resource conservation.

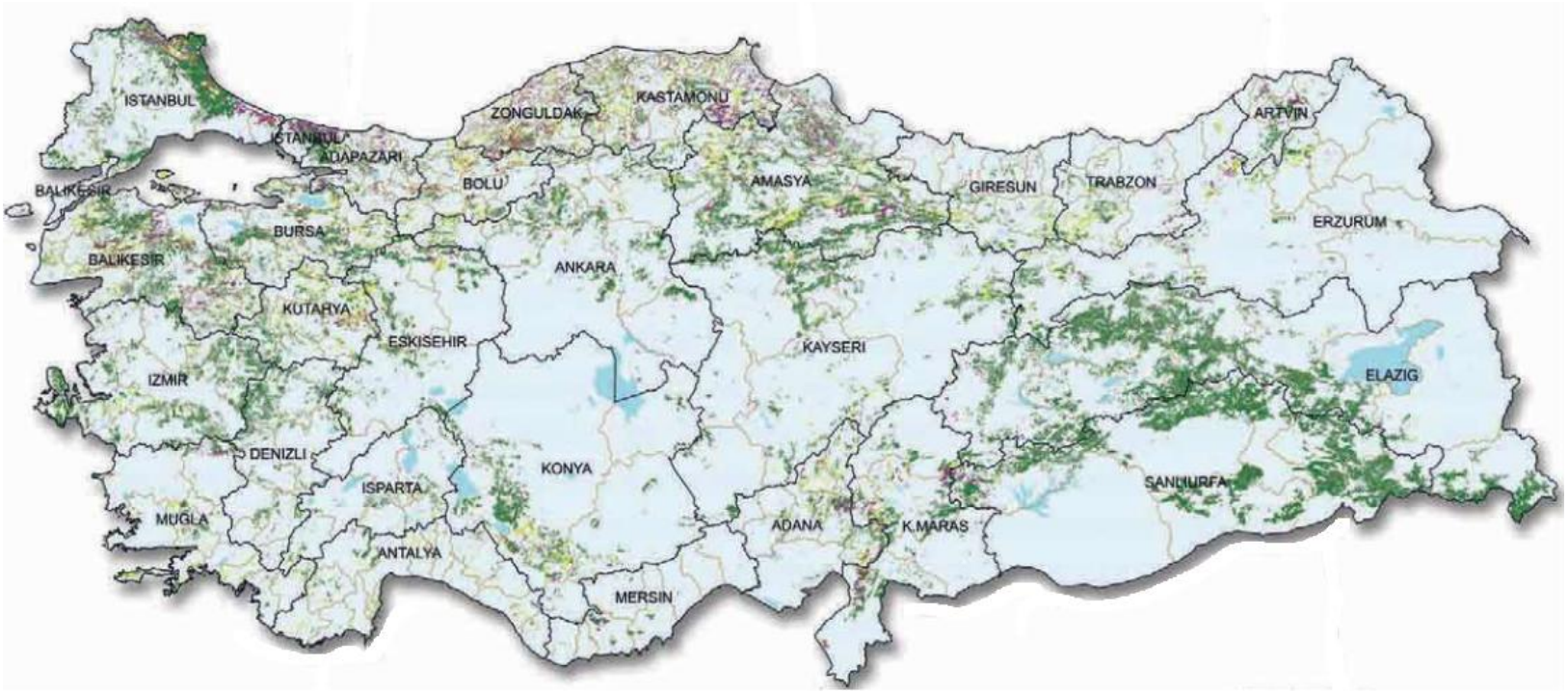


Figure 4 Distribution of oaks in Turkey

(Green: Pure oak stands; Purple: Mixtureof oaks with other species; Yellow: Mixture of other species with oaks;

Bold lines: Regional forest directorate border; Weak lines: District forest directorate border)

(Forest Atlas, 2012)

Table 4 Distribution of oaks in Turkey

(Rearranged according to Kaya and Raynal, 2001)

Type of floristic region	Geographic region	Forest types and species	Elevational range of oak species
Siberian	Black Sea & Marmara	**Dry oak and pine tress** ***Quercus* spp.**, *Pinus brutia*, *Pinus nigra* subsp. *pallasiana*	Up to 1500 m.
Mediterranean	Marmara	**Shrubs (maquis and garrigue) formation** ***Q. coccifera*, *Q. ilex***, *Arbutus* spp.	350 m.
	Aegean	**Aegean mountain (Oro-) forests** *Castanea sativa*, *Fagus orientalis*, *Tilia rubra*, *Corylus avellana*, *Pinus sylvestris*, ***Quercus* spp.**, *Pinus brutia*, *Pinus nigra*	Up to 1000m.
	Mediterranean	**Mediterranean mountain (Oro-) forests** ***Quercus* spp.**, *Pinus nigra* subsp. *pallasiana*, *Abies cilicica*, *Cedrus libani*, *Juniperus* spp., *Fagus orientalis*, *Carpinus orientalis*	500-1200 m.
Irano-Turanian	Inner Anatolia	**Tree steppe vegetation** *Juniperus oxycedrus*, *Juniperus excelsa*, *Pinus nigra*, ***Q.cerris***, ***Q. pubescens***	800-1500 m.
	Inner Anatolia	**Dry black pine, oak, juniperus forests** ***Quercus spp.***,*Pinus nigra subsp. pallasiana*, *Pinus sylvestris*	Up to 1200 m.
	East Anatolia	**Dry forests** ***Quercus spp.***, *Pinus sylvestris*	> 850m.
	South Anatolia	**Oak forests** ***Quercus* spp.**	> 850m.

1.2 Turkey oak (*Quercus cerris* L.)

Table 5 Taxonomy of *Q.cerris*

Kingdom	**Plantae**
Subkingdom	Tracheobionta
Superdivison	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Hamamelididae
Order	Fagales
Family	Fagaceae
Genus	*Quercus*
Species	***Quercus cerris* L.**

It is a member of the section *Cerris* of subgenus *Quercus* and is naturally distributed around the country except northeastern and eastern parts of Turkey (Davis, 1971; Hedge and Yaltırık, 1982). These trees are 25-30 metres in height and deciduous. As general characteristics of section members, fruits mature within two years (Figure 5). Trunk is greyish-white and becomes more fissured by age. The characteristic feature of this species is the numerous non-deciduous stipules wrapped around buds (Figure 7). Leaf morphology is an important biological indicator in botany and the same applies for oak species (Borazan and Babaç, 2003). *Q. cerris* leaves are morphologically very different and even on a single tree different leaf shapes can be observed (Davis, 1971). Leaves have regular and large amount of stellate hairs at the lower surface and less on the upper surface (Figure 6). Besides, section *Cerris* members have typically spinous projections at lobes' edges on their leaves (Davis, 1971; Hedge and Yaltırık, 1982). Their wood is not tough as white oak members. *Q. cerris* has a diploid chromosome number of 24 (Yılmaz et al., 2011).

There are two varieties of Turkey oak which are *Q. cerris* var. *cerris* and *Q. cerris* var. *austriaca*. Leaves having shallow lobes are characteristic of variety *austriaca* while deeply lobed leaves belongs to variety *cerris*. *Q. cerris* var. *austriaca* is a member of Euro-siberian floristic region and more often found in central and southeastern Europe getting through to northeast of Turkey in Thrace. Yet, variety *cerris* members disperse over the country except northeastern and eastern parts.

Turkey oak trees are wind pollinated, outcrossing and highly prone to hybridization as the other members of the genus. They hybridize with the species *Q. pubescens*, *Q. libani* and *Q. ithaburensis* which are natively distributed around the country, too (Davis, 1971).

Figure 5 Acorn example of *Q. cerris* var. *cerris* from Ankara-Çubuk (Photographed by Pelin Acar)

Figure 6 *Q. cerris* var. *cerris* leaves sampled from Ankara-Çubuk
(Photographed by Pelin Acar)

Figure 7 Stipules surrounding buds
(Photographed by K. J. McGinn)
(http://sppaccounts.bsbi.org.uk/content/quercus-cerris-1)

Figure 8 Turkey oak flowers
(http://www.aphotoflora.com/images/fagaceae/quercus_cerris_turkey_oak_tree_flowers_09-05-06.jpg)

1.3 Molecular Techniques and Genetic Diversity

Molecular marker techniques have been used for various aims in plant genetics. Nevertheless, the basic reason is to identify plants of interests. Then the following question occurs: why and for what purpose people want to identify plants by molecular techniques. The answers are various: to ease the selection of plants having desired traits and to prevent any contamination from outside, to detect plant genotypes for desired characteristic, reduce temporal and monetary losses in agricultural activities and for forensic analysis of a crime scene for example. Identification may also be important for food processors such as wine and beer producers to pick a certain type of plant variety. Detection of genetic diversity among plants is another aim. It is important for conservation studies as well as classifying them in terms of genotypic structures (Henry, 1999). There have been

different techniques used to reveal genetic relatedness of genotypes, populations and species (Stern et al., 2008). Alternative forms of an ezyme, enzyme restricted DNA products which are in different lengths, amplification of DNA parts and the synthesis of new strands, the combination of DNA synthesis and enzyme digestion are all different ways and techniques used to detect genetic diversity. Not only nuclear genomes have been used in these researches. Extranuclear genomes either chloroplastic or ribosomal codes have also been used to define diversity of plants especially at species level (Henry, 1999). As a result of progress in molecular taxonomy, the studies have even been concentrated at even the level of nucleotides (Hartl and Clark, 2007) and direct sequencing of genome parts have become a common way in phylogenetic analysis, lately (Henry, 1999).

All of these molecular analyses methods give us data at allele and even nucleotide level and provide information about genetic structure of organisms of interest (Labate, 2000). By multivariate methods, multiple datasets of molecular techniques, some of which were explained above, have been used to classify genotypes, populations or species. Cluster analysis is one of the multivariate methods. Unweighted Pair Group Method with Arithmetic Mean (UPGMA) which is a hierarchical and distance based cluster analysis technique succesively combine grouping units starting from the smallest one, till a tree or dendrogram is obtained. In these studies, whatever the method is selected, data are acquired from different sources such as morphological, biochemical, protein and DNA (Mohammadi and Prasanna, 2003). There are various softwares which propose different analyses methods including multivariate methods and statistical analyses. They give a wide range of statistical results and genetic distances may differ slightly according to the chosen programme (Labate, 2000).

1.3.1 Simple Sequence Repeats (SSR)

Tandem repeats in DNA can be classified into two groups according to their repeating unit numbers. These are minisatellites and microsatellites.

Minisatellites which were firstly discovered in humans are also called Variable Number Tandem Repeats (VNTR) (Jeffreys et al., 1985). They are 15 to 70 basepair in length and highly polymorphic in repeat unit numbers (Jones et al., 1997).

Simple sequence repeats (SSR) or microsatellites is another type of tandemly repeating DNA sequences (Staub et al., 1996; Jones et al. 1997; Henry, 1999). They are multiple repeating units of 1 to 5 nucleotides (Staub et al., 1996; Powell et al. 1996; Jarne and Lagoda, 1996; Jones et al. 1997). These repeats show a genome-wide distribution and can be placed in either genes or non-coding regions of the nuclear genome or else in extranuclear genomes (Powell et al. 1996; Jarne and Lagoda, 1996; Varshney et al. 2005).

There are two different names used for microsatellites: STRs (Simple Tandem Repeats) and SSRs. STR is the name of microsatellites found in vertebrates and SSR is used to define plant microsatellites (Staub et al., 1996). Microsatellites can be classified as genic and genomic SSRs (Varshney et al., 2005). Genic SSRs are produced from sequence data or expressed sequence tags (EST). They are specific to transcribed regions of the genomes. However, genomic SSRs can be synthesized from either transcribed or nontranscribed regions of the genomes (Varshney et al., 2005). Dinucleotide repeat markers are abundant (Staub et al., 1996; Jarne and Lagoda, 1996) and their presence was initially stated in eukaryotic genomes (Hamada et al., 1982). The most frequent dinucleotide repeats are $(AT)_n$, $(GA)_n$and $(AC)_n$, $(TC)_n$ in plants and humans respectively (Staub et al., 1996; Jarne and Lagoda, 1996). However, trinucleotide repeats are more abundant in genic SSRs (Jarne and Lagoda, 1996; Varshney et al., 2005). Another way in the classification of microsatellites is based on repeat units' structure. According to organization of repeating nuclotides, there are three types of SSRs: pure, compund and interrupted (Jarne and Lagoda, 1996). Pure SSRs have all the same nucleotide types in repeating units while compound repeats have another type of repeating unit as forming complex with another type. In interrupted SSRs, another type of repeating nucleotides are settled into the other by regular intervals. It is indicated that pure repeats' polymorphism is higher than compound and interrupted ones (Jarne and Lagoda, 1996).

As a polymorphism detection way, SSRs' first usage was by Weber and May in humans (Weber and May, 1989). Today, it is a commonly preferred and valuable marker system in various organisms. This is due to their codominant, highly polymorphic, reproducible and abundant nature (Powell et al. 1996; Jones et al., 1997; Varshney et al. 2005). It's a PCR-based method and its application is easy (Jones et al., 1997). The principle is the detection of polymorphisms arising from variable copy number of repeating units (Powell et al. 1996; Jones et al., 1997; Henry, 1999). The working principle of SSRs as polymorphism detection was illustrated in the Figure 9.

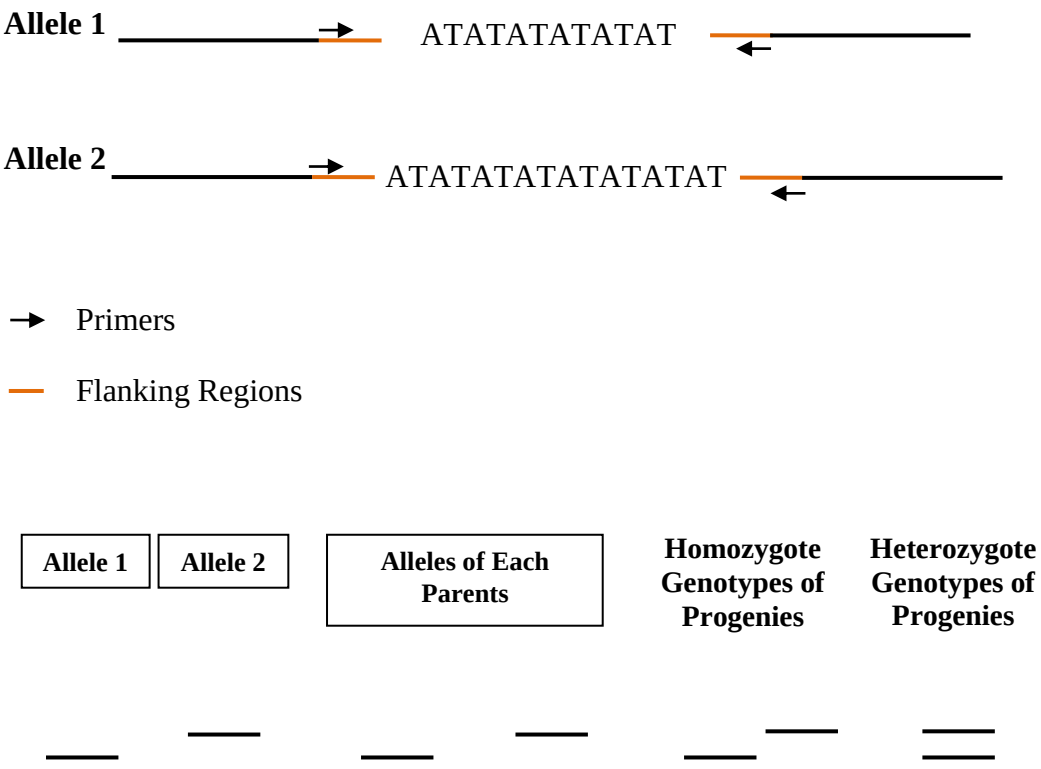


Figure 9 Working principle of simple sequence repeat primers based on length polymorphism of repeating units

On the other hand, development of SSRs is the major disadvantage of the technique (Powell et al., 1996; Jones et al., 1997; Henry,1999). Primers are designed for adjacent regions to tandemly repeated sequences which are unique and necessary to be known (Staub et al., 1996; Powell et al., 1996; Jones et al., 1997; Henry, 1999). The procedure of generating SSR primers includes several steps. These are constructing small insert genomic libraries, control for SSR having inserts, sequencing of these inserts presenting positive results, designing SSRs according to sequence data and checking if they are working (Powell et al., 1996; Jarne and Lagoda, 1996; Jones et al., 1997). Another way is directly use sequence data supported by publicly available databases which comprises gene sequences and complementary DNA (cDNA) libraries (Nunome et al. 2003a; Nunome et al. 2003b; Varshney et al. 2005).

With a fast evolving feature, microsatellites have an important role in molecular taxonomy studies even in species which show low genetic diversity especially crop species (Powell et al., 1996; Henry, 1999). SSR markers can be used in mapping and cultivar identification studies, germplasm conservation efforts and hybridity determination work (Powell et al., 1996; Jarne and Lagoda, 1996; Jones et al., 1997).

1.4 Taxonomic, Natural Hybridity and Genetic Studies in Oak Species

1.4.1 Reproductive and Chemical Studies in Genus *Quercus* L.

Despite of great morphological diversity, there are limited number of morphological features (fruit and flower) that are basically important and used in taxonomy of Fagaceae family and the genus *Quercus* (Kaul, 1985; Ducousso et al., 1993; Manos et al. 1999; Manos et al. 2001). So far, these reproductive system traits and the general indicators used for genera and subgenera discriminations were well defined in detail (Kaul, 1985; Nixon, 1993). In addition to inadequate morphological data in such a complicated taxonomy, oaks are highly interfertile which makes taxonomic efforts much more difficult. Due to these reasons and owing to advances in

molecular techniques, scientists have started to use molecular data as an important supplementary source in oak systematics, too. For example, in the study of Manos et al. (1999), genus *Quercus* subgenus *Quercus* which is the most problematic taxa of the family and the genus was analyzed with molecular data. Data obtained from nuclear and extranuclear DNA analyses was evaluated seperately and in combination to clarify the phylogeny. In another study, Manos and colleagues (2001) were tried to understand the evolution of reproductive system in 179 taxa of Fagaceae family by ITS region sequence variation. Ducousso and colleagues (1993) explicitly defined reproductive system elements and breeding system and their effects on gene flow among oaks.

In additon, there are studies dealing with chemical composition of *Quercus* species to support data to the taxonomy (Li and Hsaio, 1973; Knops and Jensen, 1980; Santamour, 1983; Guttman and Weight 1989). In these studies, isozymes and phenolic compunds were analyzed to predict natural hybridization levels. Nevertheless, neither isoenzymes nor phenolic compounds have very reliable results and the sensible feature of chemical compounds to environmental conditions makes them less useful (Rushton, 1993). DNA polymorphism is another way of detecting hybridity and has been gradually used in researches since 1990's in genus *Quercus* (Rushton, 1993).

1.4.2 Natural Hybridity Studies in Genus *Quercus* L.

Some of the studies have focused on hybridization and introgression, and their effects on phylogeny in oaks (Rushton, 1993; Muir and Schlötterer, 2005; Muir and Schlötterer, 2006; Lexer et al., 2006; Conte et al., 2007; Curtu et al., 2007; Burgarella et al. 2009). As an example, Rushton (1993) explained certain morphological criteria for hybrids' detection and mentioned about some examples. It was also emphasized that environmental conditions and any changes in them may affect hybrid formations (Rushton, 1993). Though, in another study, alterations in environmental conditions were given as one of the reasons for asymetrical introgression in *Q. cerris* and *Q. suber* (Conte et al., 2007). In the same study, two

molecular approaches (STRUCTURE and NEWHYBRIDS) were compared for suitability for the detection of a hybrid species of *Q. cerris* and *Q. suber* (Conte et al., 2007). Burgarella and co-workers (2009) interrogated the importance of molecular method selection to detect hybridity and compared two statistical softwares for their accuracy and power in *Q. suber* and *Q. ilex* hybrids' detection and concluded that STRUCTURE software gave better estimates of hybrid included analysis. In the study of Curtu et al. (2007), four species from subgenus *Quercus* and white oaks group from Romania where they co-exist, and integrate were searched for hybridity among them. It was emphasized that just morphological intermediacy could not be a sign of hybrid genotypes and levels of *F1*'s are low indeed in nature (Curtu et al., 2007).

In another study about interfertile nature of oaks, shared ancestral polymorphism among two oak species: *Q. robur* and *Q. petraea* was favored to gene flow (Muir and Schlötterer, 2005). According to microsatellite analyses and morphological seperation, it was claimed that high genetic differentiation with high frequency of shared alleles was due to shared ancestral polymorphism rather than homogenizing effect of gene flow among possibly hybridizing species (Muir and Schlötterer, 2005). This idea was opposed in the comment by Lexer et al. (2006) and interpreted that high genetic variation found among these two species could be the nature of preferred molecular technique, microsatellites. Also, it was stated that previous papers about natural or successful artifical hybridizations in oaks for several species were referred for the Lexer and colleagues's study (2006). However, in the comment of Muir and Schlötterer (2006) to Lexer et al. (2006), they re-emphasized their ancestral variation among *Q. petraea* and *Q. robur* due to high frequency of shared alleles as combined with no indication to genetic homogenization at bordering area due to high proportion of gene flow.

1.4.3 Phylogenetic and Genetic Studies in Genus *Quercus* L.

1.4.3.1 Studies on Subgenus *Quercus* Section *Quercus*

Due to their wide and historic distribution in the northern hemisphere and being the largest genus of the family, there are a lot of studies dealing with the members of the genus *Quercus* (Kaul, 1985; Nixon 1993; Manos et al., 1999). Especially the subgenus *Quercus* members have been worked molecularly to support data to its problematic systematics. So far, rather than section *Cerris* members, section *Quercus* members especially *Q. robur*, *Q. petraea*, *Q. pubescence* and *Q. frainetto* species which dominates European forests have been studied extensively (Steinkellner et al., 1997a; Kampfer et al., 1998; Barreneche et al., 1998; Dumolin-Lapègue et al., 1999; Bruschi et al., 2000; Petit et al., 2002; Bordács et al., 2002; Barreneche et al., 2004; Dzialuk et al., 2005; Curtu et al., 2007; Craft et al., 2007; Neophytou et al., 2008). In these studies, different molecular techniques were used to investigate genetic diversity and check for molecular markers' transferability to other species as well as to generate new methods for oak samples.

In the studies of Kampfer et al. (1998) and Steinkellner et al. (1997a) new microsatellite markers were generated for *Q. robur* and *Q. petraea*. In the Steinkellner and colleagues' study (1997a), the usefullness of SSR markers in three different genera members were checked. Seventeen microsatellite loci were tested for their transferrability in *Quercus*, *Fagus* and *Castanea* which were represented by eight different species in total. According to the results, all SSR markers were found amplified in all species. That was an important information of cross species application of SSR markers in oaks and for population genetic studies. In another research, Neophytou and his colleagues (2008) checked for microsatellites' transferrability for *Q. infectoria* ssp. *veneris* samples from Cyprus to support data to its taxonomy. In two comprehensive researches, phylogeny of white oaks were evaluated according to haplotype analysis of chloroplast DNA (Petit et al., 2002; Bordács et al. 2002). Four oak species with their related taxa from eight different countries in Balkans and eight species from all around Europe except a few countries were analysed in the studies of Bordács et al. (2002) and Petit et al. (2002). In another study, both mitochondrial and chloroplast DNAs from three white oak species: *Q. robur*, *Q. petraea*, *Q. pubescens* and with a relatively less number of samples from *Q. pyrenaica* were evaluated (Dumolin-Lapègue et al., 1999). According to the results, intra and interspecific gene flow in oaks were detected as species specific since the introgression is a common phenomenon in oaks (Dumolin-

Lapègue et al., 1999). In a different research, *Q. geminata* samples collected from a crime scene were investigated. Genotypes were tried to be matched to find possible potential crime scene. They were compared molecularly by microsatellites primers which were originally designed for *Q. petraea* and *Q.macrocarpa*. According to the results, microsatellites' application in forensic botany were defined as informative with an increased number of markers specific for several tree species. Also, the importance of sample collection without any comtamination was emphasized (Craft et al., 2007). In another study, Dzialuk and friends (2005) tried to find a way to decrease the cost of microsatellite analysis as multiplexing primers for *Q. petraea* and *Q. robur* samples. It was emphasized that this technique could be used in other *Quercus* species, too (Dzialuk et al., 2005). A noteworthy work is about triploidy in oaks (Dzialuk et al.,2007). In that research, *Q. petraea* and *Q. robur* samples from a mixed forest in Poland were analyzed by SSR markers and triploid *Q. petraea* genotypes were detected (Dzialuk et al., 2007). In addition to these researches based on European samples, there are also studies focusing on Asiatic oak species belong to subgenus *Quercus* (Kanno et al.,2004; Zhang et al., 2006; Ueno et al., 2008; Lee et al., 2010). Microsatellite loci development specific to Asiatic species were the aim of most of these studies.

1.4.3.2 Studies on Subgenus *Quercus* Section *Ilex*

Evergreen oak members have been studied in relatively less number of studies compared to section *Quercus* of oaks (Toumi and Lumaret 2001; Lumaret et al., 2002; López-Aljorna et al., 2007; Burgarella et al., 2009). In the study of Lumaret et al. (2002), *Quercus ilex* (Holm oak) which is the most widely found member of evergreen oaks in Europe was worked on. They investigated genetic diversity of Holm oak sampled from whole Mediterranean area by RFLP method (Lumaret et al., 2002). In a similar study, *Q. suber* (Cork oak) samples were analyzed by ISSR and SSR markers to be helpful for management study and elite tree selection activities in Spain. Since it is a raw material in wine industry, Cork oak protection, reforestration and rejuvenation is important (López-Aljorna et al., 2007). Another research was about hybrid formation between these two species: Cork and Holm Oaks (Burgarella et al., 2009). In a more comprehensive work, four evergreen oak species *Q.*

coccifera, *Q. suber*, *Q. ilex* and *Q. alnifolia* which is endemic to Cyprus were sampled from whole distribution area and compared genetically by allozyme analyses (Toumi and Lumaret, 2001).

1.4.3.3 Studies on Subgenus *Quercus* Section *Cerris*

There are relatively less number of investigation about section *Cerris* of white oaks. In some recent studies, though, *Q. cerris* has started to be incorporated into sampling and identification efforts (D'Emerico et al., 1993; Corona et al., 1995; Albert et al., 1998; Borazan and Babaç, 2003; Bellarosa et al., 2005; Muir and Schlötterer 2005; Curtu et al., 2006; Conte et al., 2007; Özcan, 2007; Yılmaz et al., 2011). A considerable amount of these researches were carried out by Italian samples (D'Emerico et al., 1993; Corona et al., 1995; Bellarosa et al., 2005; Conte et al., 2007). In these studies, hybridization among *Q. cerris* and *Q. suber* (Conte et al., 2007), chromosome analysis of the genus including Turkey oak (D'Emerico et al., 1993), relationship between climatic factor and *Q. cerris* (Corona et al., 1995) and a general study about Italian *Quercus* species were investigated (Bellarosa et al., 2005). In a molecular study of two *Quercus* species *Q. robur* and *Q. petraea*, Turkey oak was included as an outgroup (Muir and Schlötterer, 2005). In the study of Albert et al., fresh acorns of *Q. cerris* were phytochemically analyzed for detection of measurable formaldehyde levels (1998). Curtu and colleagues (2006) added Turkey oak samples to genetic analysis of four most studied oak species *Q. robur*, *Q. petraea*, *Q. frainetto* and *Q. pubescens*. In the study, three type of molecular markers (microsatellites, cpDNA-RFLP and isozymes) were applied. According to results, *Q. cerris* was found the most genetically distinct species from the rest of the species (Curtu et al., 2006).

To date, studies about *Q. cerris* sampled from Turkey are much less in amount and were about their morphology, karyomorphology and biochemistry (Borazan and Babaç, 2003; Özcan, 2007; Yılmaz et al. 2008; Yılmaz et al. 2011). In Yılmaz and colleagues' study (2011), karyotype analysis of oaks from *Ilex* and *Cerris* sections were analyzed. Chromosome numbers were compatible with chromosome number of

the genus. In another work, leaf morphology of five oak species which were *Q. robur*, *Q. petraea*, *Q. pubescens*, *Q. virgiliana* sampled from Bolu province of Turkey were used to find differentiation among them (Borazan and Babaç, 2003). In the study by Özkan (2007), 16 *Quercus* taxa of Turkey were examined according to the fatty acid compositions of mature acorns. It was proposed that acorn oils may be a useful tool to support data to taxonomy of *Quercus* at infrageneric level. Major oil types detected from all taxa were oleic, linoleic, palmitic, alpha linoleic and stearic acids. According to this study, the varieties of Turkey oak, *Q. cerris* var. *cerris* and *Q. cerris* var. *austriaca*, show little variation for total oil content and only the difference was found in palmitic acid amounts. This difference was defined for within variety level and may be used as a variety index. It was established that linoleic, alpha linoleic and oleic acids sectional mean amounts are higher in both *Q. cerris* varieties than other members of section *Cerris*. Also notable, general fatty acid profiles were found similar for *Q. cerris* and *Q. pubescens* which hybridize.

CHAPTER 2

JUSTIFICATION OF THE STUDY

- *Q. cerris* (Turkey oak) is a native member of Turkey forests. Total of 6.4 million ha of Turkey, constituting 76.4% of broadleafed forests is covered by oak species (Çolak and Rotherham, 2006; Forest Atlas, 2012). However, these forests are faced with a serious degradation.

- The taxonomy of genus and especially subgenus *Quercus* including *Q. cerris* is problematic. It is due to great morphological diversity and high tendency to hybridization among species generating intermediate morphology. Due to these facts, species in the genus and subgenus has been extensively studied. However, there is little known about the genetic diversity of *Q. cerris* and the other oak members sampled from Turkey.

The studies about Turkey oak are limited. The species has been incorporated into the studies only recently as an outgroup member (Muir and Schlötterer, 2005; Curtu et al., 2006). As a major component of Turkey forests, there are few researches (Borazan and Babaç, 2003; Özcan, 2007; Yılmaz et al. 2008; Yılmaz et al. 2011) but no genetic data about Turkey populations of *Q. cerris*. When considering degradation rates and speed, the composition of these populations needs to be investigated for conservation and management of genetic resources of Turkey oak (Kaya and Raynal, 2001; Çolak and Rotherham, 2006; Şekercioğlu et al., 2011). In this study, genetic structure of *Q. cerris* populations and the genetic diversity among them is designated.

CHAPTER 3

OBJECTIVES OF THE STUDY

The objectives of this study are;

- To determine the magnitude and pattern of genetic diversity of Turkey oak (*Q. cerris*) populations sampled from the natural range of the species in Turkey by using SSR markers.

- To assess the effects of suspected hybrid genotypes at sampling areas where Turkey oak trees coexist and naturally hybridize with other *Quercus* species (*Q. libani*, *Q. brantii*, *Q. trojana*, *Q. infectoria*, *Q. pubescens*, *Q. ithaburensis*).

- To generate genetic diversity data that will be useful for establishing principles for conservation and management of genetic resources of Turkey oak forests.

CHAPTER 4

MATERIALS AND METHODS

4.1 Materials

4.1.1 Plant Materials

The plant materials used in this study were collected from four different oak species rich regions of Turkey covering 10 *Q. cerris* populations and three different *Q. cerris* populations for additional sampling. Total 13 populations were evaluated within the scope of the project, TOVAG 1080723 "Molecular phylogeny of Turkish oaks: Evolutionary relationships and the role of natural hybridization in speciation". The locations of populations with their coordinates are listed in Table 6.

Sampling survey was done in an area elevation-based from top to down and in horizontal plane. It was aimed to collect minimum twenty samples from twenty different spots by hundred meters intervals between each individual. However, due to some unavailabilities both in field sampling and during DNA extraction efforts, sufficient number of genotypes could not be collected for each population location.

Fresh leaves of Turkey oak trees were collected during field trips. Except the region Kırklareli, each region had at least one suspected hybrid individual. Kahramanmaraş-Adıyaman, Konya-Isparta and Kastamonu regions comprised the largest number of suspected hybrids among all regions. Members of other species which were *Q. infectoria*, *Q. libani*, *Q. brantii*, *Q. pubescens* and *Q. trojana* were included in analyses. These materials were sampled from the same regions during field trip, as well. All studied populations' locations were given in Table 6 with their coordinates and shown on the map in Figure 10. Four oak rich regions were indicated by stars in

Table 6. All studied *Q. cerris* and suspected hybrid genotypes were given with their locations and number of genotypes in each location in Table 7.

Table 6 Description about *Q. cerris* distribution in each population site in Turkey

CODES		LOCATIONS and COORDINATES			
		AFYON-ESKİŞEHİR			
	1 SUN	Sultandağ-Sündiken	38°16’25’’N 39°55’54’’N	31°24’04’’E 31°08’16’’E	1202m-1327m
		ANKARA*			
	2 CUB	Çubuk	40°21’23’’N- 40°32’24’’N	32°54’49’’E- 32°97’63’’E	1198m-1504m
		BALIKESİR			
	3 DUS	Dursunbey	39°34’35’’N 39°34’40’’N	28°25’11’’E- 28°25’13’’E	559m-582m
		KAHRAMANMARAŞ-ADIYAMAN*			
	4.1 BOZ	Boztoprak	37°32’14’’N 37°32’22’’N	36°16’05’’E 36°16’47’’E	908m-951m
	4.2 ERC	Erince	37°43’40’’N 37°48’10’’N	37°24’19’’E 37°25’29’’E	1131m-1566m
4	**4.3 TRL**	Tırıl	31°35’48’’N 37°36’21’’N	36°18’28’’E 36°19’81’’E	1234m-1396m
	4.4 ADI	Gölbaşı	37°34’18’’N 37°34’55’’N	37°28’99’’E 37°29’40’’E	926m-987m
		KASTAMONU*			
5	**5.1 KAST**	Kayalı	41°31’54’’N 41°32’24’’N	33°45’26’’E 33°45’44’’E	1113m-1192m
	5.2 KOY	Küre	41°32’49’’N 41°45’22’’N	33°37’47’’E 33°44’30’’E	1095m-1245m
		KIRKLARELİ			
6	**IDA**	İğneada-Sisliada	41°50’21’’N 41°54’01’’N	27°47’47’’E 27°54’16’’E	19m-428m
		KONYA-ISPARTA*			
	7.1 ISP	Yalvaç-Eğirdir	38°13’17’’N 38°13’17’’N	31°17’13’’E 31°17’53’’E	1262m-1500m
7	**7.2 BUL**	Bulcuk	38°09’19’’N 38°10’06’’N	31°55’23’’E 31°55’26’’E	365m-1418m
	7.3 CAT	Çatak	38°09’18’’N 38°10’31’’N	31°51’57’’E 31°54’02’’E	-

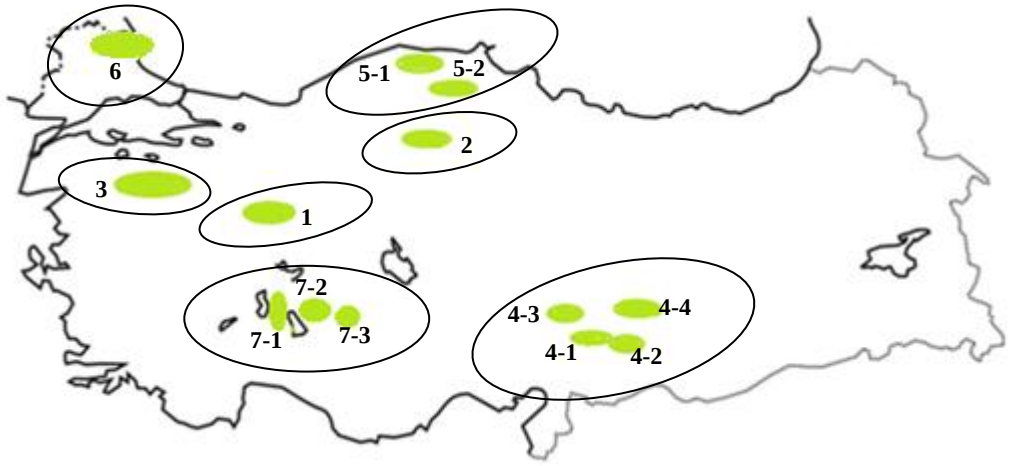


Figure 10 Map showing the locations of the studied *Q. cerris* populations

4.1.2 Sample DNA Extraction

Leaves collected during field trip were stored in bags containing silica gel pellets. Leaf samples were grinded in mortar by pestle with liquid nitrogen and powdered ready for DNA extraction procedure.

Genomic DNA from powdered leaf materials was extracted according to the Doyle & Doyle method (1987) with a few modifications. At first step, to each 20 ml of CTAB (cetyl trimethylammonium bromide) extraction buffer, 0.8 gram polyvinylpyrrolidone (PVP) was added. This buffer was than heated in water bath at 60°C for 15 minutes till PVP dissolves ready to use in DNA extraction. Leaf powders were added to tubes and ground by total 1500 µl CTAB solution including PVP in two-stages. Then to each tube, 100 µl β-mercaptoethanol and 5 µl proteinaz K were added and mixed gently. Samples were held in water bath for 1 hour andmeanwhile shaked occasionally. After 1 hour, tubes were centrifuged at 14000 rpm (resolutions per minute) for 20 minutes. Supernatants were taken into new 1.5 ml tubes and 500 µl chloroform-isoamyl alcohol (24:1) was added to each sample.

Next, samples were centrifuged at 14000 rpm for 15 minutes to seperate the phases. Supernatants were collected and transferred to new tubes and if necessary, that procedure repeated once more. 600 µl cold isopropanol was added to tubes in order to precipitate DNAs. Additional to original procedure, cold isopropanol added tubes held in -80°C overnight. Then, samples were again centrifuged at 14000 rpm for 20 minutes. Aqueous phases were spilled and pellets were washed out by 500 µl ethanol twice. Afterwards, tubes were allowed to dry until whole ethanol evaporated in room temperature. To resuspend DNA pellets, 60 µl TE buffer or distilled water were added.

Then, the quantity of genomic DNA was assessed by Thermo NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific Inc. NanoDrop 2000 Spectrophotometer Version 1.4.1). According to the quantity surveys, the amounts of DNA were adjusted to 10 ng/µl to use in further experiments.

Table 7 Sampling information on *Q. cerris* and suspected hybrid genotypes

(Numbers given below the locations' codes refer to the codes indicated in Table 6)

Species and Hybrids	**Locations and Size of Populations**															
	SUN 1		**CUB 2**	**DUS 3**	**BOZ 4-1**	**ERC 4-2**	**TRL 4-3**	**ADI 4-4**	**KAST 5-1**	**KOY 5-2**	**IDA 6**		**ISP 7**		**BUL 7-2**	**CAT 7-3**
	Sultandağ	Sündiken	Çubuk	Dursunbey	Boztoprak	Erince	Tırıl	Gölbaşı-Haydarlı	Kayalı	Küre-Oyrak	İğneada	Sisliada	Eğirdir	Yalvaç	Bulcuk Yolu	Çatak
Q. cerris var. *cerris*	4	3	8	12	13	7	12	16	19	22			12	1	16	16
Q. cerris var. *austriaca*											9	2				
Q. cerris X *Q. libani*						11		3								
Q. cerris X *Q. brantii*						4										
Q. cerris X *Q. trojana*														1		1
Q. cerris X *Q. infectoria*						1		7	7							1
*Q. cerris*X *Q. pubescens*		1	1						3						2	2
Q. cerris X *Q. ithaburensis*				2												

4.2 Methods

4.2.1 SSR Primers and the Optimization of PCR Conditions

At the begining of the study, 20 different SSR primers, which were designed for several *Quercus* species, were selected. These primers were checked for PCR amplification status whether they work and give polymorphic bands or not. For this aim, several PCR profiles and alternative amounts of components in reactions were tested and products were checked by agarose gel electrophoresis. 3% agarose gel prepared by 1x TBE (Tris-Borate-EDTA) solution were used to check DNA. Samples were run for at least 30 minutes at ~120 mA. In order to visualize DNA bands in gels, 5 μg/ml ethidium bromide was used. At the last step, these gels were visualized by AlphaImager Gel Documentation System (Alpha Innotech, San Leandro, CA, USA) (Figure 11).

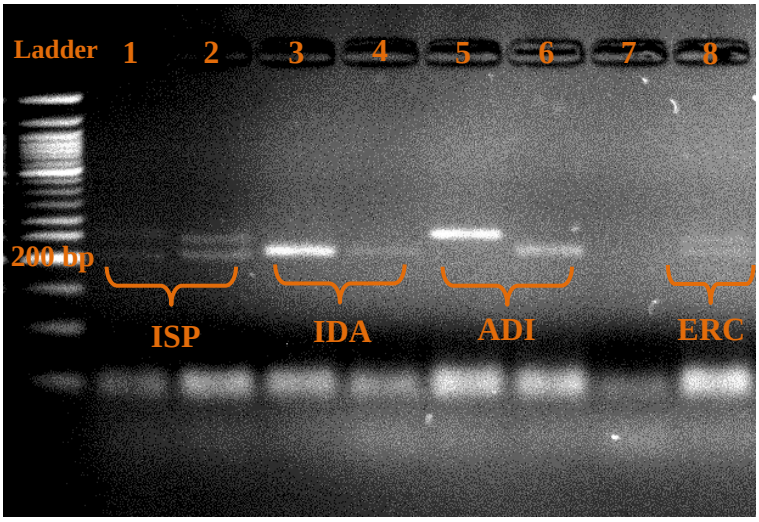


Figure 11 The figure shows the PCR products of ssrQpZAG36 locus for seven genotypes from different populations

(Lines 1 to 2: Genotypes from Isparta population; 3 to 4: Genotypes from İğneada population; 5 to 6: Genotypes from Adıyaman population; 8: Genotype from Erince population)

After these preliminary experiments, 12 SSR loci qualified for further studies were selected. Selected primers for SSR loci were listed in Table 8 with their sequences, repeat units and melting temperatures. The amounts of components in PCR reaction mixture and the best PCR profiles were given in Table 9 and Table 10, respectively.

Table 8 Repeat motifs and sequences of SSR loci

Locus	**Forward primer (5'-3')**	**Backward primer (3'-5')**	**Repeat unit**	**T (°C)**	**Reference**
ssrQpZAG9	GCAATTACAGGCTAGGCTGG	GTCTGGACCTAGCCCTCATG	(AG)12	52	Steinkellner et al. 1997a
ssrQpZAG36	GATCAAAATTTGGAATATTAAGAGAG	ACTGTGGTGGTGAGTCTAACATGTAG	(AG)19	52	Steinkellner et al. 1997a
ssrQrZAG102	GCCTACACTCTTCAATCTACATGA	GACTTGTAACACCTTAAGCATTATCT	(GA)29	53	Kampfer et al. 1998
QmC01758	GGCACGAGGTTTCTTTGCTTAGAC	CCCACCAGTACTTTGGGCTTACC	(GGA)6	55	Ueno et al. 2008
QmC00716	AAGAGAACCCATTCCATCCCTGA	TTTCCCGAACAGTGGTTTCTTGA	(TC)15	55	Ueno et al. 2008
QmC00932	AGGCTCAAAACAAAACCAAACCG	CCCCTTTCCCATAATCAAACCCT	(TC)19	55	Ueno et al. 2008
QmC00963	TGAAGCCTGCGTTAACAACAACA	TTCTCCTCCTCCTCGCTACTCGT	(GAT)6	55	Ueno et al. 2008
MSQa3	TGACCTATAAGCTTCATGTACG	TCAACCTAGCAAGATTCCTC	(AG)27	60	Lee et al. 2010
MSQa8	ATACGTCCTGTTGAGATGA	TGCCACTCCCTACCGTCAG	(AG)25	56	Lee et al. 2010
MSQa10	ATGGCTGTATGAATAGTACTC	TGACTAGATGACTGCAATGAG	(GT)11	52	Lee et al. 2010
MSQa11	ATGTGACCTTGAACACGGG	TTAAAGGCCTAGAGGCTAG	(GT)11	56	Lee et al. 2010
MSQa14	TTTAGTAATTGGCGTTAAAG	ACTGGCAATTATCTAAAATC	(AG)17	52	Lee et al. 2010

Table 9 Optimized PCR conditions for the studied primers

Primer Name	**DNA (10ng/µl)**	**PCR Buffer (10X)**	**$MgCl_2$ (25mM)**	**dNTP (10mM)**	**Primer (10µM)**	**Taq (5u/µl)**	**H_2O**	**Total (µl)**
ssrQpZAG9	4	5	4	1	0.5+0.5	0.5	9.5	25
ssrQpZAG36	4	5	4	1	0.5+0.5	0.5	9.5	25
ssrQrZAG102	3	4	2	0.5	0.5+0.5	0.5	14	25
QmC01758	3	4	2	0.5	0.5+0.5	0.5	14	25
QmC00716	4	5	4	1	0.5+0.5	0.3	9.7	25
QmC00932	4	5	4	1	0.5+0.5	0.3	9.7	25
QmC00963	4	5	4	1	0.5+0.5	0.3	9.7	25
MSQa3	4	5	4	1	0.5+0.5	0.3	9.7	25
MSQa8	4	5	4	1	0.5+0.5	0.5	9.5	25
MSQa10	4	5	4	1	0.5+0.5	0.5	9.5	25
MSQa11	4	5	4	1	1 + 1	0.5	8.5	25
MSQa14	4	5	4	1	0.5+0.5	0.5	9.5	25

Table 10 PCR profile cycles

	PCR condition 1			**PCR condition 2**		
Denaturation	94 °C	5'		94 °C	2'	
	94 °C	30"	30 cycles	95 °C	30"	35 cycles
Annealing	55°C	25"		56°C	45"	
	72 °C	30"		72 °C	1'	
Final extension	72 °C	15'		72 °C	10'	

4.2.2 Data Collection

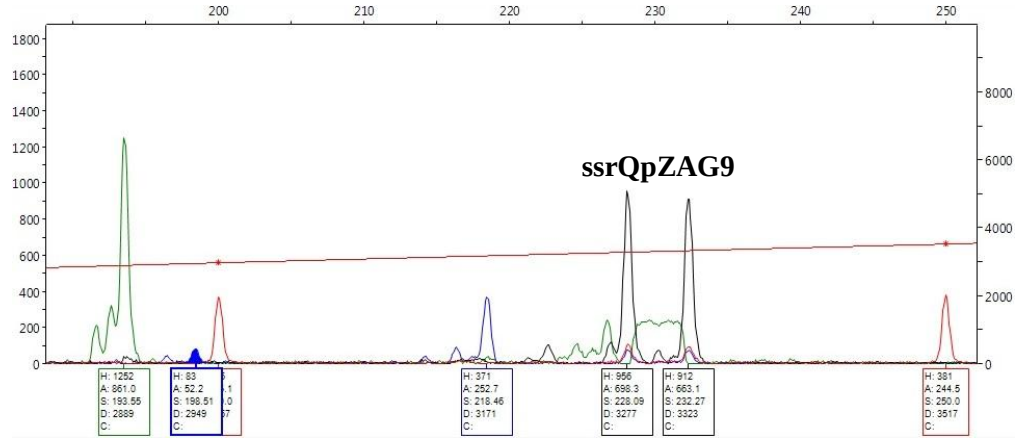
After optimization of primers and PCR protocols, either forward or reverse primer of each selected SSR primer pairs was resysnthesized fluorescently to be detected in genetic analyzer system. Three different coloured dyes were used to end-label primers: Fam, Tamra and Hex. DNAs were amplified by these fluorescent primers. The primer products were combined due to economical and work load concerns. It was designed as four SSR primers' products that were in three different colours were analyzed for one individual at one time. The important point was to consider sizes of primers' alleles. In order to prevent confusion of PCR products which were close in size, those primers were labeled by differently coloured fluorescent dyes. On the other hand, some dye signals were dominant and suppressing others would appearin the sequencer. So, to be able to observe all the results in combinations, PCR products of loci which were monitored by dominant dyes were used in less amounts in mixtures. For better understanding, SSR primers used in experiments were listed according to types of dye and alleles' size ranges in developed species in Table 11.

Table 11 Three combinations of twelve SSR primers used in experiments

	Locus	Flourescent Dye			Allele Size Range
		Tamra	Hex	Fam	
Combination 1	ssrQpZAG9	✓			182-210
	ssrQpZAG36			✓	210-236
	ssrQrZAG102		✓		195-276
	QmC01758			✓	368-386
Combination 2	QmC00716			✓	245-275
	QmC00932		✓		233-259
	QmC00963	✓			264-298
	MSQa3			✓	327-359
Combination 3	MSQa8		✓		191-221
	MSQa10	✓			331-343
	MSQa11		✓		253-271
	MSQa14			✓	260-278

Assay procedure was performed by the REFGEN Biotechnology Company (Teknokent, METU, Ankara) with an ABI 310 Genetic Analyzer (PE Applied Biosystem) automatic sequencer for microsatellite analysis. During analysis, colourful polymorphic bands of alleles were obtained as peaks due to their sizes. Visualization was done by Peak Scanner Software v1.0 software, 2006 (http://www.appliedbiosystems.com). In this software, Sizing Default NPP (NPP: No Primer Peaks) was selected as analyzing method while size standart was GS500. Polymorphic bands were scored as 1 or 0 which was based on presence or absence of alleles. This data were then re-formatted in a file to be used in further genetic analysis. Examples of band polymorphisms between the genotypes from Kahramanmaraş populations were indicated by arrows in Figure 12 and 13.

ERC 1 – *Q. cerris* var. *cerris*



ERC 43 – *Q. cerris* var. *cerris*

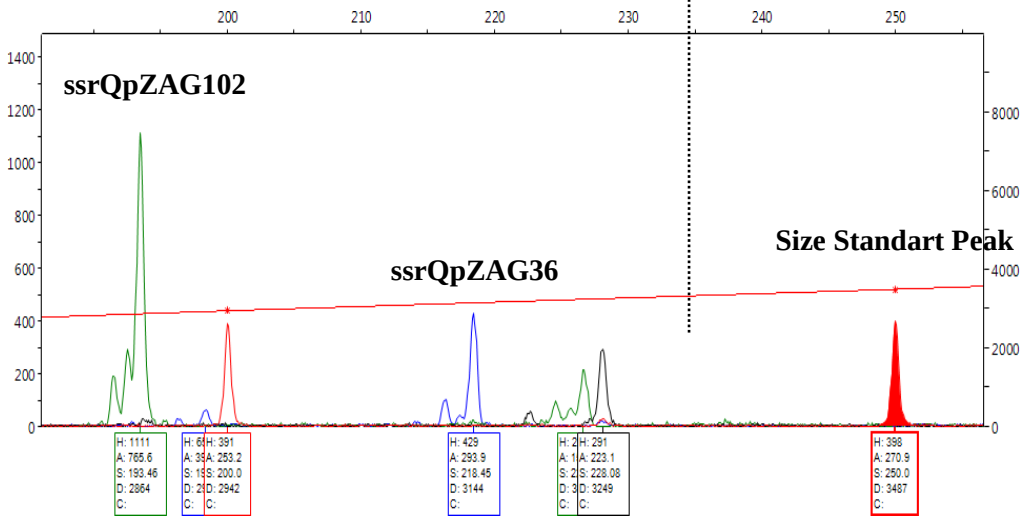
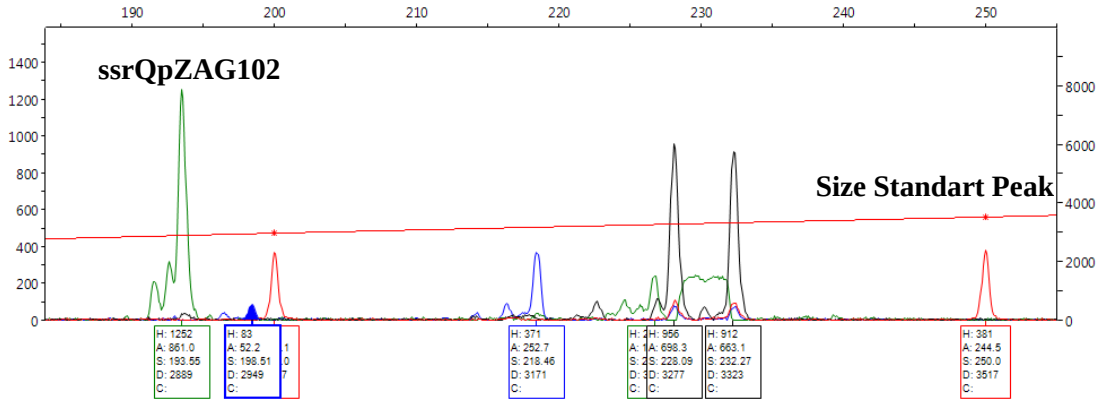
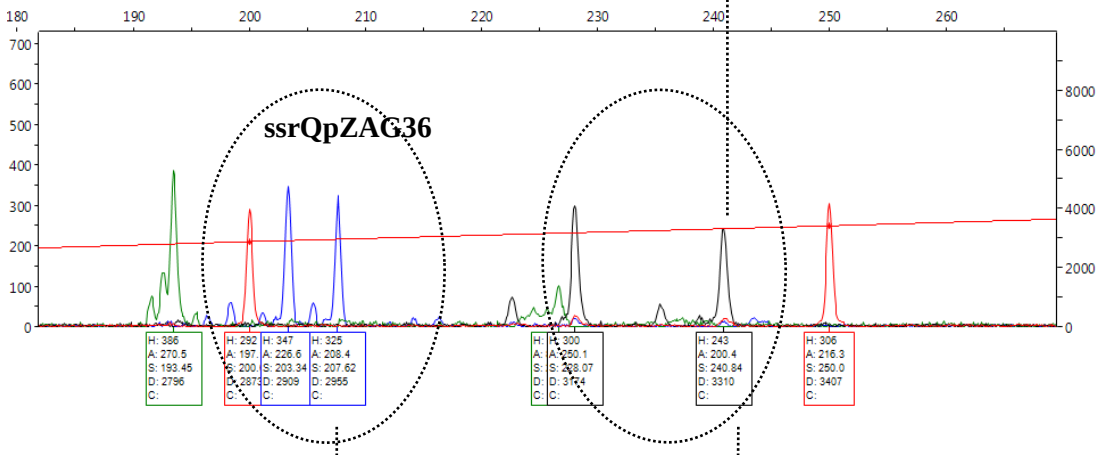


Figure 12 The peak profiles of two genotypes from Erince population for ssrQpZAG9, ssrQpZAG36 and ssrQpZAG102 loci

ERC 1 – *Q. cerris* var. *cerris*



ERC 62 – *Q. cerris* var. *cerris* X *Q. libani*



TRL 127 – *Q. libani*

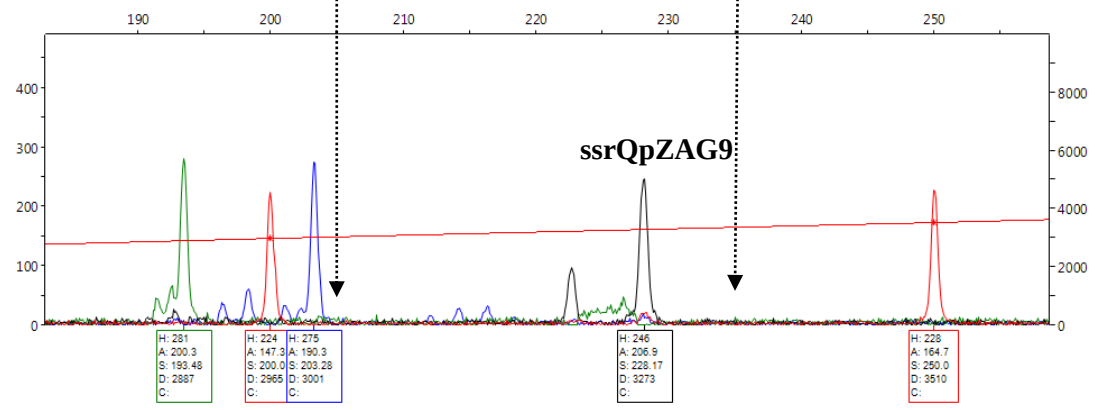


Figure 13 The peak profiles of three genotypes from Kahramanmaraş populations for ssrQpZAG9, ssrQpZAG36 and ssrQpZAG102 loci

4.2.3 Analysis of Data

In order to understand genetic relationship of Turkey oak populations, data obtained by primer peaks' evaluation was used for statistical analysis. By these analyses, genetic data was used to interpret diversity and gather information about inbreeding, heterozygosity of populations and to infer the true number of populations etc. Data was analyzed seperately with suspected hybrid genotypes in populations or without them. Suspected hybrid genotypes included assay was searched in a more detailed way that the regions rich in these genotypes were examined seperately in PCA. Samples from other species; *Q. infectoria, Q. libani, Q. brantii, Q. pubescens, Q. trojana* were included in these analyses. These materials had been sampled from the same regions during field trip. A series of programmes were used to analyze the data for several purposes.

GDA (Genetic Data Analysis) was used to estimate; mean number of alleles per locus (A), mean number of alleles per polymorphic locus (Ap), the proportion of polymorphic loci (P), expected (He) and observed (Ho) heterozygosity values (Lewis and Zaykin, 2001). *F*- Statistics (Wright, 1951) were also computed by the programme. A genetic tree demonstrating relationship of populations according to coancestry identity (Nei, 1978) was drawn by UPGMA method.

STRUCTURE software (Version 2.3.4., 2012) was also utilized for the analysis of Turkey oak genotype data. STRUCTURE is used to designate genotypes to populations and find the most probable number of populations according to allele frenquencies (Pritchard et al., 2000). It is a model-based Bayesian clustering approach and has two models: no admixture and with admixture. Assumptions of modelling is based on the origin of genotypes whether they are from a single population and have a common ancestry or have a mixed ancestry (Pritchard et al., 2000; Flaush et al., 2007). Clustering of genotypes is achieved by MCMC (Markov Chain Monte Carlo) algorithm and the number of MCMC replication, length of burnin period and number of replication for each run are all related to the update of parameters. By this update, accuracy for the constitution of clusters (populations)

increased (Pritchard et al., 2000; Flaush et al., 2007). In this study, default parameters were set. Admixture model was selected and allele frequencies were defined as correlated among populations. Admixture alpha was to be inferred from the dataset and lambda values was assigned 1.0 as proposed in the programme manual (Pritchard et al., 2010). The number of clusters (*K*) defined from 1 to 13. Structure was run for 100,000 iterations of MCMC replication a burn-in period of 10,000 iterations. Each run was replicated 20 times as *K*=1 to 13.

By STRUCTURE software, a tree is constructed demonstrating the genetic distances among inferred clusters according to the allele-frequency distances among clusters. It is constituted by Neighbor Joining Method (Saitou and Nei, 1987) and the plot is constituted according to DRAWTREE (Felsenstein, 2005).

The output file of Structure software gives the estimation of probability distribution (Pr *X // K*) which observed as "Ln P(D)" in the file (Evanno et al., 2005). The log likelihood of data computed for each of MCMC run and so, we get the same number of "Ln P(D)" as the iteration number for each *K* value. Thereafter, mean of these values should be taken and half their variance should be substracted to the mean to reach *L(K)* (Evanno et al., 2005). The maximal value of *L(K)* is defined as the exact number of clusters (Evanno et al., 2005). However, ad hoc procedure was proposed to obtain better estimation of true *K* (*ΔK*) (Pritchard et al., 2000) which was explicitly explained by Evanno et al. (2005). The following sequence of calculations which are differences between successive likelihood values should be done to reach *ΔK* value are:

L(K) = An average of values (preferred iteration number; equal to 20) of *Ln P(D)*

$$L'(K) = L(K) - L(K - 1)$$

$$|L''(K)| = |L'(K + 1) - L'(K)|$$

$$\Delta K = m(|L''(K)|)/s[L(K)]$$

CLUMPP (Cluster Matching and Permutation Program, Version 1.1.2) and Distruct (Version 1.1) were also used for Turkey oak microsatellite data analysis. The output file of STRUCTURE arranged and becomes the raw data for CLUMPP and its output is used as an input file for Distruct (Rosenberg, 2004; Jakobsson and Rosenberg, 2007). CLUMPP is an optimization program of cluster analysis outcomes which may show differences in replicates at different times. In other words, it tries to find the best alignment of cluster analysis data (Jakobsson and Rosenberg, 2007). Distruct is a visualization program of clusters (subgroups) that are constitued due to coefficient membership of genotypes. It is a graphical presentation and offers a variety of options for figures' display (Rosenberg, 2004).

GENEPOP and POPGENE were the softwares used for population genetics analysis (Raymond and Rousset, 1995; Population Genetic Analysis POPGENE Version 4.2). In the GENEPOP software, Hardy-Weinberg Exact Tests, Population Differentiation and *Fst* & Other Correlations options were applied to *Q. cerris* data through the internet. Estimation of exact P-Values was performed in the option of Hardy-Weinberg Exact Tests by the Markov Chain Method. Markov Chain parameters for all tests were chosen as dememorization number: 1,000, the number of batches: 100 and the number of iterations per batch: 1,000. In the population differentiation option, genotypic differentiation for all populations were calculated by default parameters of Markov Chain parameters. Allele frequency-based correlation (*Fis*, *Fit* and *Fst*) values for each locus were calculated in the option *Fst* & Other Correlations and compared with GDA results. In the POPGENE software, co-dominant marker data of SSRs were used as raw data to achieve results. Populations' expected and observed heterozygosities as well as observed and effective number of alleles were calculated with their standart deviations.

PCA (Principle Component Analysis) was utilized to determine potential hybrid's situation in three dimesional plane by NTSYS-pc Version 2.2j programme (Rohlf, 2008). To do this, a matrix of correlations among variables was computed by Simint module in the software. Presence and absence microsatellite data was the input file for this step. Pearson product moment correlation was the coefficient for calculations. The outfile of this module was the input of Eigen module. Eigen values

representing principle components and two and three dimensional plots of genotypes were obtained. It is referred that the Principle Component Analysis (PCA) can be used with a combination to clustering results (Mohammadi and Prasanna, 2003). It may also give an idea about genotypes or populations which have an intermediate characteristics as long as most of the variation is explained in first axes (Lessa, 1990). For these reasons and in order to evaluate the genetic proximity between *Q. cerris* and other oak species, which coexist, hybridize and form mixed oak stands together, and to determine their positions in three dimensional planes, PCA analysis were conducted. First, the positions of populations without any suspected hybrid genotypes were determined. Then, the regions which are rich in oak species were evaluated individually. For the regional analyses, three different PCA were carried out.

CHAPTER 5

RESULTS

5.1 SSR Primers, Polymorphism and Statistics

All of the primers which were chosen for this thesis works after preliminary studies were worked well and gave polymorphic results. Among studied SSR primers, just MSQa11 worked with PCR condition-2 which was given in Table 10. All remaining primers worked with PCR condition-1.

Among the screened 20 SSR primers which were developed for various oak species, 12 of them were successfully amplified and yielded polymorphisms. The defined allele size ranges of microsatellite loci developed for species of interests were given in Table 12. Product size ranges represent the same primers' products and detected number of alleles were the total allele number of primers for *Q. cerris* sampled in Turkey (Table 12).

The loci which had the highest mean number of alleles per locus were QmC00932, MSQa10 and MSQa14. The least values of this parameter though were observed in ssrQrZAG102 and MSQa8 loci. Remaining seven loci mean number of alleles were changed from 10 to 15 (Table 12).

Table 12 Microsatellite loci details

Locus	**Derived from**	**Allele size range**	**Product size range**	**Detected number of alleles**
ssrQpZAG9	*Q. petraea*	182-210	185-263	15
ssrQpZAG36	*Q. petraea*	210-236	198-203	12
ssrQrZAG102	*Q. robur*	195-276	194-240	5
QmC01758	*Q. mongolica* var. *crispula*	368-386	335-379	12
QmC00716	*Q. mongolica* var. *crispula*	245-275	236-259	15
QmC00932	*Q. mongolica* var. *crispula*	233-259	215-257	23
QmC00963	*Q. mongolica* var. *crispula*	264-298	270-300	10
MSQa3	*Q. acuta*	327-359	308-375	13
MSQa8	*Q. acuta*	191-221	185-209	6
MSQa10	*Q. acuta*	331-343	313-359	22
MSQa11	*Q. acuta*	253-271	230-266	13
MSQa14	*Q. acuta*	260-278	239-279	22

According to statistical results in the absence of suspected hybrid genotypes, all studied loci were highly polymorphic (100%). The highest number of private allele was detected in ssrQpZAG9 and the least one in MSQa8. The mean A and Ap values were found 12.58. The lowest values for the mean number of alleles were detected in MSQa8 and ssrQrZAG102 loci. Nevertheless, the highest values were observed in MSQa14 and QmC00932. The expected heterozygosities for each locus varied between 0.36 and 0.93 with a mean value of 0.73. The mean observed heterozygoty was 0.53 for all studied populations. Locus MSQa8 had the lowest Ho value (0.08). The ssrQrZAG102 and ssrQpZAG9 were the only loci which had observed heterozygosity values higher than expected ones. All these statistical results were given in Table 13. According to the estimation of exact P-Values performed by the Markov chain method, F_{is}, F_{it} and F_{st} values were found highly significant for all loci and all populations. However, for the ssrQpZAG9, QmC00716, MSQa8 and MSQa11 loci, F_{is} and F_{it} values were detected nonsignificant. All loci F_{st} s' were found statistically highly significant at $P<0.001$ (Table 13).

Table 13 Descriptive and *F* statistics results of GDA programme based on loci data without suspected hybrid genotypes (P= Proportion of polymorphic loci, A= Average number of alleles per locus Ap= Average number of alleles per polymorphic locus, Ho= Observed heterozygosity, He= Expected heterozygosity, *Fis*= Inbreeding coefficient within a subpopulation, *Fit*= Overall fixation index, *Fst*= Fixation index ** Statistically significant at P<0.05, *** Statistically highly significant at P<0.001)

Locus	**Private Allele No**	**P**	**A**	**Ap**	**Ho**	**He**	***Fis***	***Fit***	***Fst***
ssrQpZAG9	7	1	12	12	0.70	0.65	-0.21	-0.06	0.12***
ssrQpZAG36	2	1	11	11	0.65	0.78	-0.08**	0.18**	0.24***
ssrQrZAG102	2	1	5	5	0.40	0.36	-0.43**	-0.07**	0.25***
QmC01758	3	1	11	11	0.62	0.84	-0.03***	0.29***	0.31***
QmC00716	3	1	13	13	0.44	0.76	-0.03	0.45	0.47***
QmC00932	3	1	21	21	0.73	0.92	0.13**	0.21**	0.09***
QmC00963	1	1	9	9	0.55	0.75	0.20**	0.26**	0.08***
MSQa3	2	1	10	10	0.49	0.66	-0.29**	0.28**	0.44***
MSQa8	0	1	4	4	0.08	0.49	0.26	0.85	0.80***
MSQa10	2	1	20	20	0.55	0.89	0.30***	0.40***	0.14***
MSQa11	2	1	13	13	0.58	0.78	-0.04	0.28	0.31***
MSQa14	2	1	22	22	0.61	0.93	0.30***	0.34***	0.06***
Mean	**29**	**1**	**12.58**	**12.58**	**0.53**	**0.73**	**0.04*** **	**0.28*** **	**0.25*** **

According to statistical results with suspected hybrid genotypes, all loci were highly polymorphic (100%). The highest number of private allele was detected in ssrQpZAG9 and the least one in MSQa8 and in QmC01758. The mean of A and Ap values were found 13.83. The lowest values for mean number of alleles were 4 and 5 as they detected in MSQa8 and ssrQrZAG102 loci. Nevertheless, the highest value was 23 which was observed in QmC00932. The second highest value was 22 which was split up in two primers; MSQa10 and MSQa14. The expected heterozygosities for each locus varied between 0.41 and 0.93 with a mean of 0.77. The observed heterozygoties' mean value was 0.53. Locus MSQa8 had the lowest Ho value (0.08) and showed a significant difference to rest of loci. The ssrQrZAG102 and ssrQpZAG9 were the only loci which had observed heterozygosity values higher than expected ones. All these statistical results were given in Table 14. According to the estimation of exact P-Values, F_{is}, F_{it} and F_{st} values were found highly significant for all loci and all populations.

Table 14 Descriptive and *F* statistics results of GDA programme based on loci with suspected hybrid genotypes

Locus	**Private Allele No**	**P**	**A**	**Ap**	**Ho**	**He**	***Fis***	***Fit***	***Fst***
ssrQpZAG9	7	1	15	15	0.70	0.69	-0.10**	-0.003**	0.09***
ssrQpZAG36	2	1	12	12	0.67	0.79	-0.06***	0.17***	0.21***
ssrQrZAG102	1	1	5	5	0.42	0.41	-0.26***	0.02***	0.22***
QmC01758	0	1	12	12	0.62	0.86	0.08***	0.30***	0.24***
QmC00716	1	1	14	14	0.46	0.83	0.16***	0.47***	0.37***
QmC00932	5	1	23	23	0.72	0.92	0.16***	0.22***	0.07***
QmC00963	1	1	10	10	0.56	0.78	0.24***	0.29***	0.07***
MSQa3	4	1	14	14	0.47	0.73	0.06***	0.37***	0.34***
MSQa8	0	1	4	4	0.08	0.53	0.63***	0.86***	0.63***
MSQa10	2	1	22	22	0.53	0.91	0.34***	0.42***	0.11***
MSQa11	2	1	13	13	0.56	0.83	0.11***	0.34***	0.25***
MSQa14	1	1	22	22	0.62	0.93	0.28***	0.33***	0.06***
Mean	**26**	**1**	**13.83**	**13.83**	**0.53**	**0.77**	**0.13*** **	**0.31*** **	**0.21*** **

5.2 Populational Genetic Parameters, Diversity Statistics and Distance

5.2.1 Results in theAbsence of Suspected Hybrid Genotypes

A total of 172 genotypes, 11 from *Q. cerris* var. *austriaca* and all remaining from *Q. cerris* var. *cerris*, were analyzed with GDA programme. According to descriptive statistics, South-Central Turkey populations were highly polymorphic as the Southeastern and Northern Turkey populations' variation was less than 100%. The proportion of polymorphic loci (P) of the Kastamonu-Küre population calculated as 0.58 which was the lowest value of all. The second lowest value was observed in Ankara-Çubuk population. The overall mean of this parameter was calculated as 0.91. The highest value of mean number of alleles (A) was observed in the populations of Konya-Çatak and Isparta. The lowest value was 2.75 and found in the Ankara-Çubuk population. The mean number of observed alleles per polymorphic loci (Ap) for all populations was 5.13 which ranged from 3.10 to 6.33. Kastamonu-Küre and Kırklareli-İğneada populations had no private alleles while Konya-Çatak and Kahramanmaraş-Erince had the most private alleles. The total private allele number for all SSR primers was 29 and 10 of them were observed in Kahramanmaraş-Adıyaman and 13 of them found in Konya-Isparta region populations. The mean expected heterozygosity value of all populations was 0.61. It changed from 0.38 to 0.73. All populations had He between 0.60 and 0.70 except Ankara-Çubuk, Kastamonu-Kayalı and Kastamonu- Küre populations. Isparta had the highest He value as Kastamonu-Küre had the lowest. The mean observed heterozygosity was detected as 0.56 which was lower than the mean expected heterozygosity value. The lowest Ho and He values were calculated for Kastamonu and Ankara populations. All these statistical results were given in Table 15.

Table 15 Population genetic parameters of *Q. cerris* populations without suspected hybrid genotypes

Population	**Codes**	**n**	**Private Allele No**	**P**	**A**	**Ap**	**Ho**	**He**	***Fis***	***Fis***	***Fit***	***Fst***
Kahramanmaraş-Erince	ERC	7	5	0.92	4.25±0.18	4.54±0.22	0.66±0.03	0.68±0.02	0.06**	-0.02	0.02	0.04
Kahramanmaraş-Boztoprak	BOZ	13	3	0.92	5.17±0.26	5.54±0.31	0.72±0.02	0.64±0.02	-0.14***			
Kahramanmaraş-Tırıl	TRL	12	1	0.92	5.50±0.22	5.91±0.26	0.71±0.02	0.67±0.02	-0.07***			
Adıyaman	ADI	16	1	0.92	5.33±0.17	5.73±0.26	0.58±0.02	0.65±0.02	0.13***			
Konya-Bulcuk	BUL	16	3	1	5.67±0.19	5.67±0.24	0.68±0.01	0.67±0.01	-0.01***	0.04	0.11	0.07
Konya-Çatak	CAT	16	7	1	6.33±0.16	6.33±0.24	0.65±0.02	0.68±0.02	0.04***			
Isparta	ISP	13	3	1	6.33±0.21	6.33±0.31	0.71±0.02	0.73±0.02	-0.02***			
Kırklareli-İğneada	IDA	11	0	1	5.20±0.14	5.20±0.21	0.56±0.02	0.63±0.02	0.12			
Eskişehir-Sündiken	SUN	7	1	0.90	4.60±0.13	5.00±0.18	0.60±0.02	0.67±0.02	0.11			
Balıkesir-Dursunbey	DUS	12	1	1	4.78±0.15	4.78±0.17	0.47±0.02	0.67±0.02	0.31***			
Ankara-Çubuk	CUB	8	2	0.83	2.75±0.08	3.10±0.13	0.32±0.03	0.41±0.02	0.13**	0.16	0.36	0.23
Kastamonu-Kayalı	KAST	19	2	0.92	4.00±0.15	4.27±0.24	0.29±0.02	0.40±0.02	0.28**			
Kastamonu-Küre	KOY	22	0	0.58	2.92±0.09	4.28±0.18	0.36±0.03	0.38±0.03	0.05***			
Mean		**172**	**29**	**0.91**	**4.83±0.47**	**5.13±0.32**	**0.56±0.01**	**0.61±0.01**	**0.05*** **	**0.05*** **	**0.29*** **	**0.26*** **

For 12 loci and 13 populations, *F* statistics parameters were estimated and given in Table 15. According to the results, the mean *Fst* value of Turkey oak populations was found as 0.26. This means that 26% of genetic variation was between populations as 74% of genetic variation was within populations. The mean inbreeding coefficient (*Fis)* of all populations was calculated as 0.05. As positive results indicate heterozygote deficiency, the overall correlation of genes within populations was found in favour of homozygotes. The mean *Fit* of 13 Turkey oak populations was calculated as 0.29. This parameter is the overall inbreeding coefficient of an individual within total population. Similarly, the positive values of F_{it} means increased homozygosity. The mean $F_{is,}$ F_{it} and F_{st} values were found highly significant for all loci and all populations. Individual F_{is} values of each population were found to be significant and highly significant except for Kırklareli-İğneada and Eskişehir Sündiken populations.

For the *F* statistics analysis, the studied populations were grouped into three big clusters to be calculated with the GDA (Table 15). This grouping was due to general observation on populations' structure and geographical proximity as well as genetic analysis results. The *F* statistics results without suspected hybrid genotypes revealed that there is a general tendency to homozygosity in terms of inbreeding coefficient values. The mean *Fis* value of all populations was 0.05. Only negative result of inbreeding coefficient was detected in Kahramanmaraş-Adıyaman populations. So, the highest heterozygote value was calculated in this zone. This means that among three big clusters, Kahramanmaraş-Adıyaman cluster was the only cluster showing excess of heterozygotes although it was not large. The group which had the highest homozygosity was Ankara-Kastamonu. The highest values of *Fis*, *Fit* and *Fst* were estimated for Ankara-Kastamonu hybrid zone as 0.16, 0.36 and 0.23, respectively.

To estimate genetic distance, distance matrix which had 13 populations was constructed. Nei's (1978) genetic identity and distance measures based on 12 SSR loci were used. By this matrix, the UPGMA tree of Turkey oak populations was constructed (Figure 14). In this dendrogram, two major groups were observed. These were Kastamonu-Ankara hybrid zone populations and the remaining Anatolian populations extending up to Thrace population. According to the distance matrix,

genetic distance between these two major groups was calculated 0.56. When the distance among the groups was calculated, the genetic difference between Ankara-Çubuk population and Kastamonu populations was found 0.13. Kahramanmaraş-Adıyaman populations and the other Anatolian populations extending up to the Thrace populations formed seperate clusters in the second major group. Distance between these two clusters was calculated 0.17. Genetically the most closer populations were İğneada-Sündiken, Bulcuk-Isparta and Erince-Boztoprak (Figure 14).

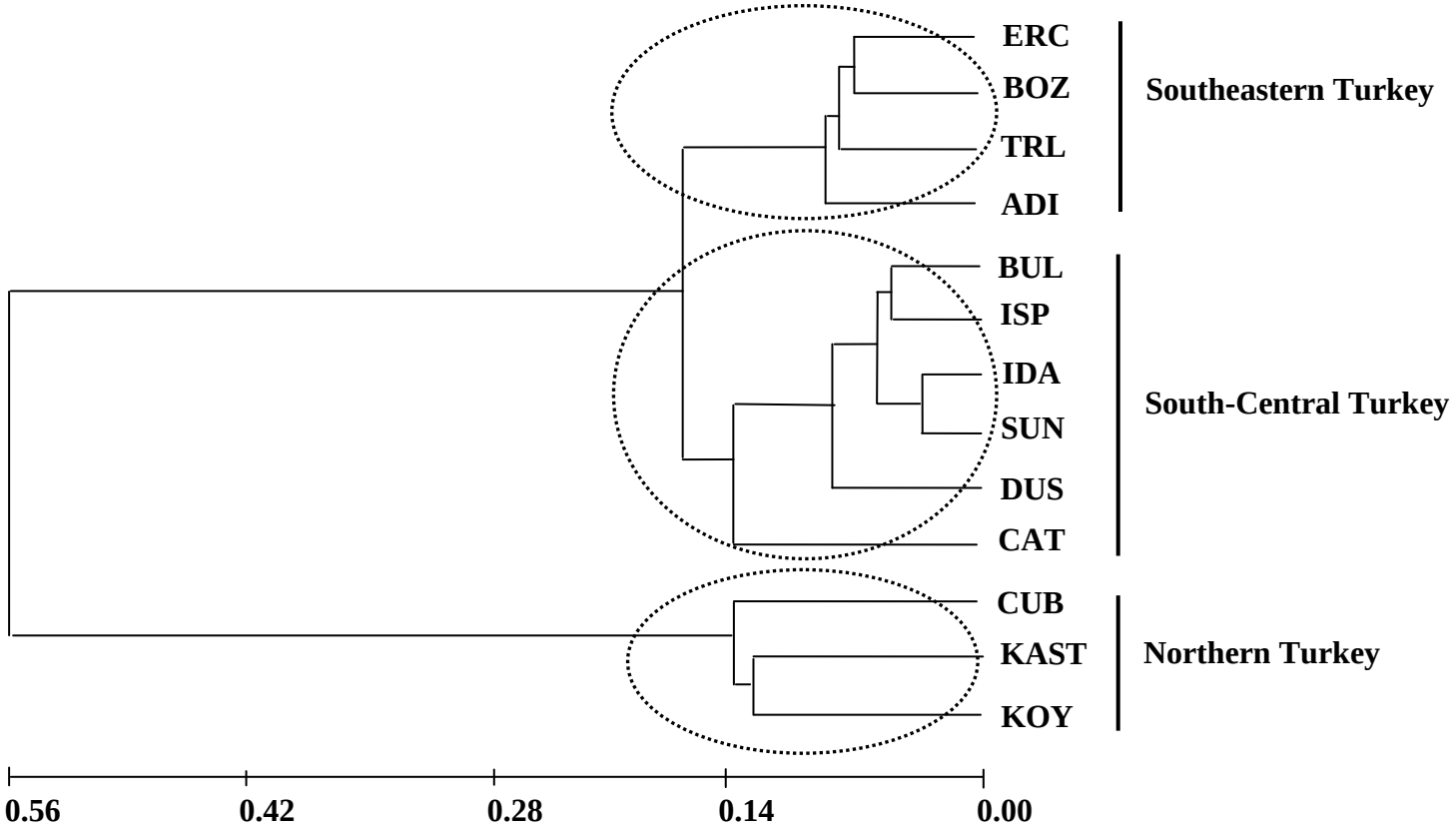


Figure 14 The UPGMA dendrogram of *Q. cerris* populations sampled from Turkey

Total 172 genotypes were analyzed by using 12 microsatellite loci. This data was run in the STRUCTURE software (Pritchard et al., 2000). A partial example of text data file run in STRUCTURE was provided in Appendix B.

Results of STRUCTURE analysis revealed that, the best *K* was inferred by delta *K* method and found as two. Each calculation step was given in table and actual population number was given in bold (Table 16). The chart of *K* and delta *K* values was given in Figure 15. The aim was to determine actual population number and to define the identity of genotypes with respect to populations that they belong to. In Figure 16, two clusters of populations grouped by population ID's were given in single plot line. It could be seen that populations from Ankara-Çubuk, Kastamonu-Kayalı and Kastamonu-Küre were clustered seperately and the remainings formed the other cluster. The detailed plot demostrating genotypes in multiple lines was also given in Appendix D.

Table 16 Inference of the best *K* using the delta *K* method

K	**LnP(D) - L(K)**	**VarLnP(D)**	**L'(K)**	**L'' (K)**	**ΔK**
1	-8787.13	146.88	/	/	/
2	-7569.45	296.23	1217.68	639.82	**2.16**
3	-6991.59	483.19	577.85	270.60	0.57
4	-6684.34	615.24	307.25	147.64	0.24
5	-6524.74	769.55	159.60	91.04	0.12
6	-6456.17	1033.95	68.565	77.90	0.07
7	-6309.71	1060.30	146.46	34.41	0.03
8	-6128.83	1009.49	180.88	73.86	0.07
9	-6021.81	1046.77	107.01	302.67	0.29
10	-6217.47	1632.59	-195.66	257.99	0.16
11	-6155.14	1759.92	62.335	728.35	0.41
12	-6821.16	3273.85	-666.02	1040.77	0.32
13	-6446.40	2791.75	374.75	374.75	0.13

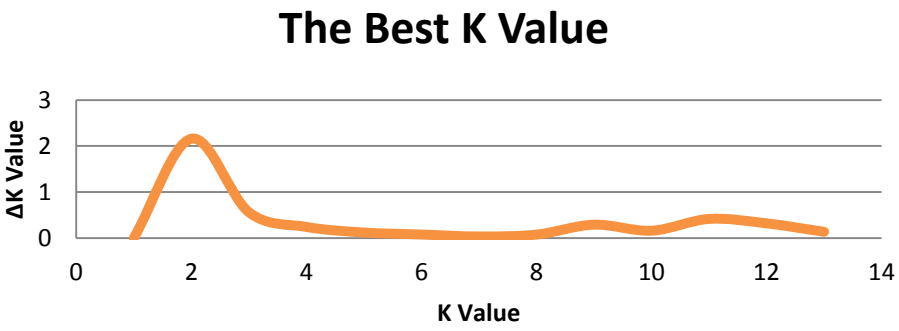


Figure 15 The maximum *K* value based on the delta *K* method

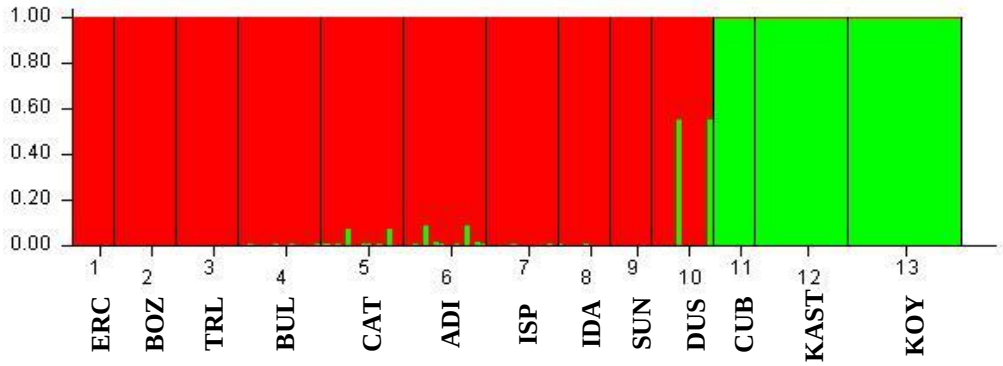


Figure 16 The plot of populations given in a single line

(Red: Southeastern and South-Central Turkey oak populations;
Green: Northern Turkey oak populations)

5.2.2 Results with Suspected Hybrid Genotypes

Total 219 genotypes with 47 suspected hybrid genotypes were analyzed with the GDA programme. Of these suspected hybrid genotypes, most of them were collected from Kahramanmaraş-Erince, Adıyaman and Kastamonu-Kayalı populations. Kahramanmaraş Boztoprak and Tırıl, Kırklareli-İğneada and Kastamonu-Küre populations had no suspected hybrid genotypes. Remainings comprised just one or two suspected hybrid genotypes.

According to descriptive statistics, the least variable populations were from Northern Turkey. The lowest value of the proportion of polymorphic loci (P) was calculated 0.58 for Kastamonu-Küre population. The overall mean of this parameter was 0.93. The highest value of mean number of alleles (A) (8.25) was observed in Kahramanmaraş-Erince population. The lowest value was 2.75 and found in Ankara-Çubuk population. The mean number of observed alleles per polymorphic loci (Ap) for all populations was 5.77 and ranged from 3.10 to 8.25. The highest and lowest values of this parameter were also detected in the Kahramanmaraş-Erince and Ankara-Çubuk populations. Six of the populations had no private alleles while Kahramanmaraş-Erince had most of the private alleles. The overall number of private alleles was 26 and 14 of them were observed in Kahramanmaraş-Adıyaman and seven of them found in Konya-Isparta region populations. The mean expected heterozygosity value of all populations was 0.62. It ranged from 0.38 to 0.74. All populations had He between 0.60 and 0.70 except for the Ankara-Çubuk and Kastamonu- Küre populations. The Kahramanmaraş-Erince population had the highest and Konya-Çatak had the second highest He values with 0.74 and 0.73, respectively. The Kastamonu-Küre population had the lowest expected heterozygosity. The mean observed heterozygosity was detected as 0.55 which was lower than the expected heterozygosity. The lowest Ho values calculated for the Kastamonu and Ankara populations. All these statistical results were given in Table 17.

According to the *F* statistics' results, the mean *Fst* value of Turkey oak populations was found as 0.21. This means that 21% of genetic variation was between populations as 79% of genetic variation was within populations. The mean inbreeding coefficient (*Fis)* of all populations was calculated as 0.14. This means that the overall correlation of genes within populations is in the favour of homozygotes. The mean *Fit* of 13 Turkey oak populations was found 0.32. This parameter is the overall inbreeding coefficient of an individual within total population. The mean *F* coefficients were found highly significant for 12 loci and all populations. Individual F_{is} values of each population were found significant except for Kırklareli-İğneada population. In Table 17, three parameters of *F* statistics were given. Also, three zones were defined according to their geographical location and proximity and for each, three *F* statistics parameters were calculated (Table 17). With respect to results, the minimum inbreeding coefficient value was detected in Kahmaranmaraş-Adıyaman zone. So, the highest heterozygote amount was in this zone. The lowest *Fit* and *Fst* belonged to this zone, too. The highest values were detected in Ankara-Kastamonu zone in terms of all *F* parameters. 0.14, 0.32 and 0.21 were the overall mean of *Fis*, *Fit* and *Fst.*

Table 17 Statistical results of GDA programme based on population data with suspected hybrid genotypes

Population	**Codes**	**n**	**Private Allele No**	**P**	**A**	**Ap**	**Ho**	**He**	***Fis***	***Fis***	***Fit***	***Fst***
Kahramanmaraş-Erince	ERC	23	12	1	8.25±0.23	8.25±0.34	0.65±0.02	0.74±0.02	0.11***	0.07	0.13	0.06
Kahramanmaraş-Boztoprak	BOZ	13	0	0.92	5.17±0.26	5.54±0.31	0.72±0.02	0.64±0.02	-0.14**			
Kahramanmaraş-Tırıl	TRL	12	1	0.92	5.50±0.22	5.91±0.26	0.71±0.02	0.67±0.02	-0.07***			
Adıyaman	ADI	26	1	1	6.08±0.22	6.08±0.31	0.52±0.02	0.65±0.02	0.21***			
Konya-Bulcuk	BUL	18	0	1	5.75±0.19	5.75±0.24	0.67±0.01	0.67±0.01	-0.003***	0.076	0.14	0.07
Konya-Çatak	CAT	20	5	1	7.50±0.17	7.50±0.26	0.67±0.02	0.73±0.01	0.08***			
Isparta	ISP	14	2	1	6.33±0.22	6.33±0.32	0.70±0.02	0.71±0.02	-0.02***			
Kırklareli-İğneada	IDA	11	0	1	5.20±0.14	5.20±0.21	0.56±0.02	0.63±0.02	0.12			
Eskişehir-Sündiken	SUN	8	0	0.91	5.27±0.17	5.70±0.22	0.55±0.03	0.67±0.02	0.19**			
Balıkesir-Dursunbey	DUS	14	0	0.90	5.10±0.16	5.55±0.19	0.42±0.02	0.60±0.02	0.32***			
Ankara-Çubuk	CUB	9	1	0.83	2.75±0.07	3.10±0.14	0.32±0.03	0.42±0.02	0.15**	0.33	0.45	0.18
Kastamonu-Kayalı	KAST	29	4	1	5.83±0.19	5.83±0.33	0.33±0.02	0.62±0.01	0.46***			
Kastamonu-Küre	KOY	22	0	0.58	2.92±0.09	4.28±0.19	0.36±0.03	0.38±0.03	0.05***			
Mean		**219**	**26**	**0.93**	**5.51±0.33**	**5.77±0.51**	**0.55±0.01**	**0.62±0.01**	**0.14*** **	**0.14*** **	**0.32*** **	**0.21*** **

To estimate genetic distance, distance matrix with the 13 taxanomic units (populations) was estimated by Nei's (1978) genetic identity based on 12 SSRs. By this matrix, the UPGMA tree of Turkey oak populations was constructed.

According to the tree, two major clusters were observed. These were Kastamonu-Ankara zone populations and Kahramanmaraş-Adıyaman and inner Anatolian populations extending up to the Thrace population. Genetic distance between these two major groups was detected 0.57 according to the distance matrix. Kahramanmaraş-Adıyaman and inner Anatolian populations formed seperate clusters. Distance between these two clusters was calculated 0.23. Genetically the most closer populations were İğneada-Sündiken, Bulcuk-Isparta and Boztoprak-Tırıl (Figure 17).

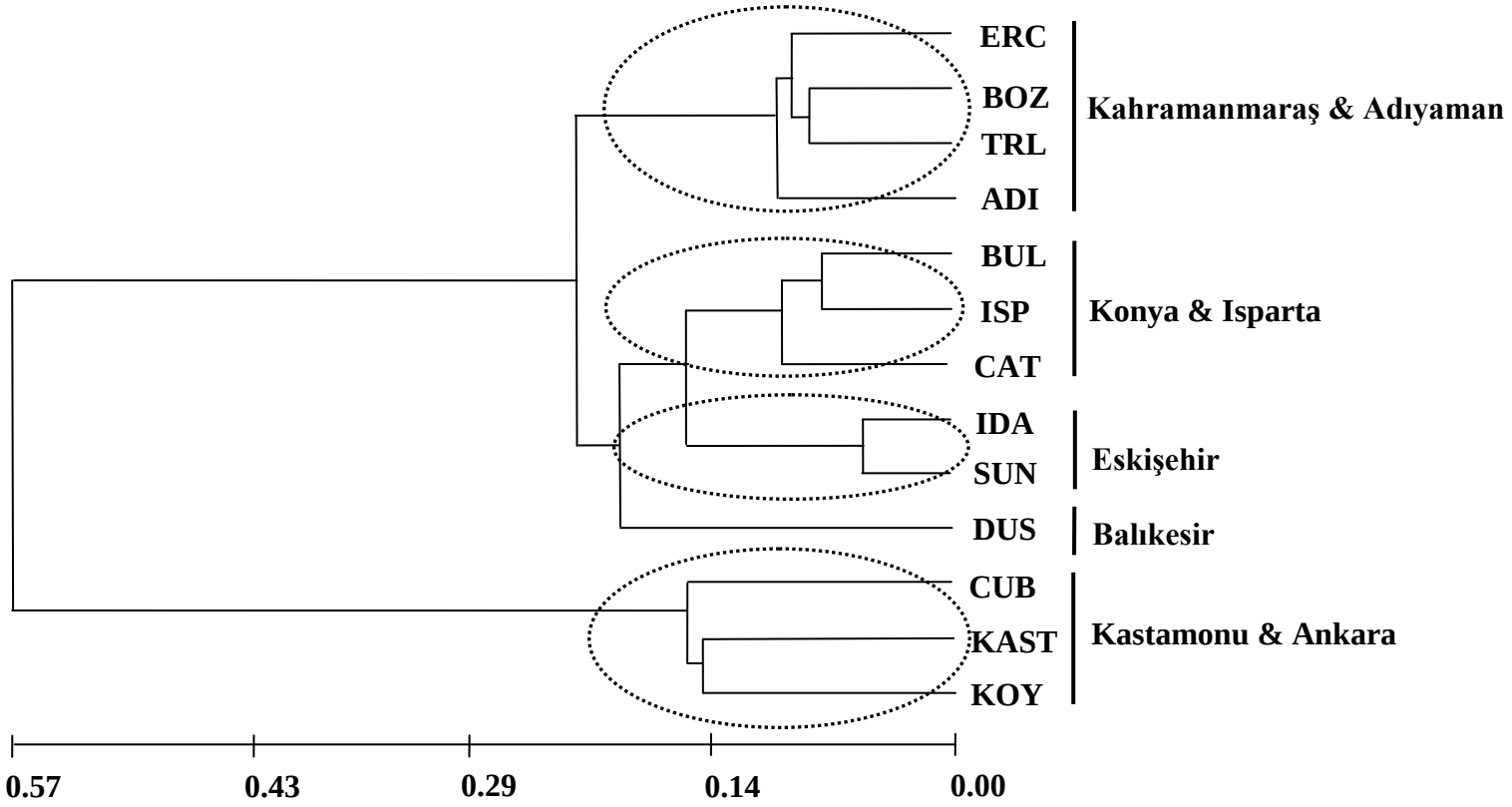


Figure 17 The UPGMA dendrogram of *Q. cerris* populations constructed with suspected hybrid genotypes

Total of 219 Turkey oak genotypes were also analyzed with the STRUCTURE software to get the real population number of analyzed Turkey oak populations. The best *K* was inferred by delta *K* method according to the outfile of software. The values of estimated probabilities of *K* and the second rate of likelihood were given in Table 18 and the chart of *K* and *ΔK* was in Figure 18.

Table 18 Inference of the best *K* using the delta *K* method for 13 *Q. cerris* populations

K	**LnP(D) - L(K)**	**VarLnP(D)**	**L'(K)**	**L'' (K)**	**ΔK**
1	-11988.46	185.82	/	/	/
2	-10596.82	423.20	1391.64	623.29	1.47
3	-9828.47	610.60	768.34	438.20	0.72
4	-9498.33	823.14	330.14	29.82	0.04
5	-9138.37	799.79	359.96	162.10	0.20
6	-8940.52	895.43	197.85	64.28	0.07
7	-8806.94	997.15	133.57	59.78	0.06
8	-8613.59	1035.63	193.35	288.30	0.28
9	-8708.53	1513.98	-94.94	50081.19	**33.08**
10	-58884.67	102037.94	-50176.14	47655.35	0.47
11	-61405.46	107415.45	-2520.79	10595.67	0.10
12	-74521.92	133892.75	-13116.46	72638.33	0.54
13	-15000.05	15125.98	59521.87	59521.87	3.93

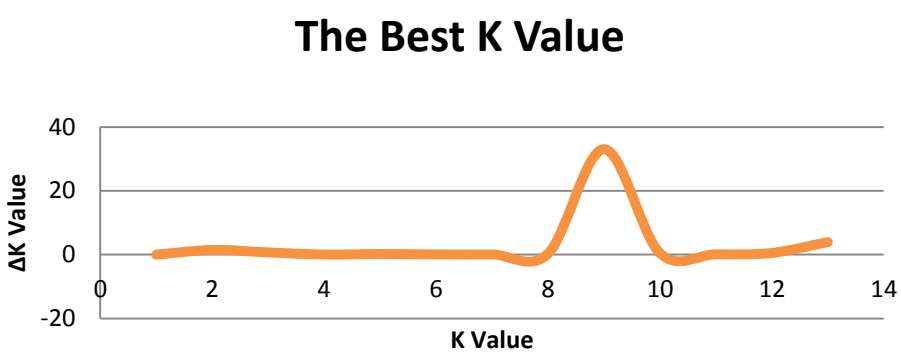


Figure 18 Graph of delta *K* and *K* values for 13 *Q. cerris* populations

In Figure 19, nine clusters of populations grouped by population ID's was given in single plot line. When the Figure 19 was observed, two big clumps were seen. These were Kahramanmaraş populations (in yellow) and Kastamonu-Ankara populations (in green). The detailed plot demostrating genotypes in multiple lines was also given in Appendix E.

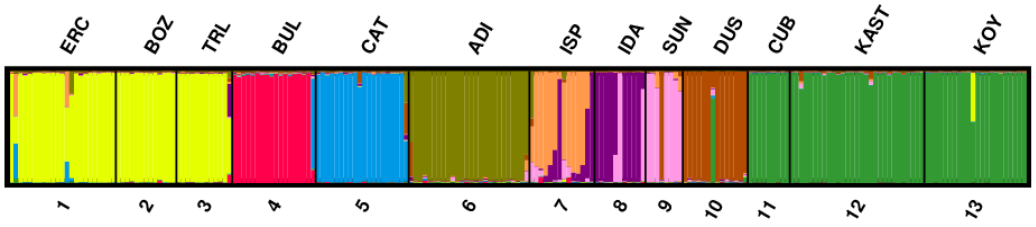


Figure 19 The plot of populations analyzed in Distruct 1.1 programme

(Yellow: The cluster of Kahramanmaraş populations; Green: The cluster of Kastamonu&Ankara populations; individual clusters of the other populations in different colours)

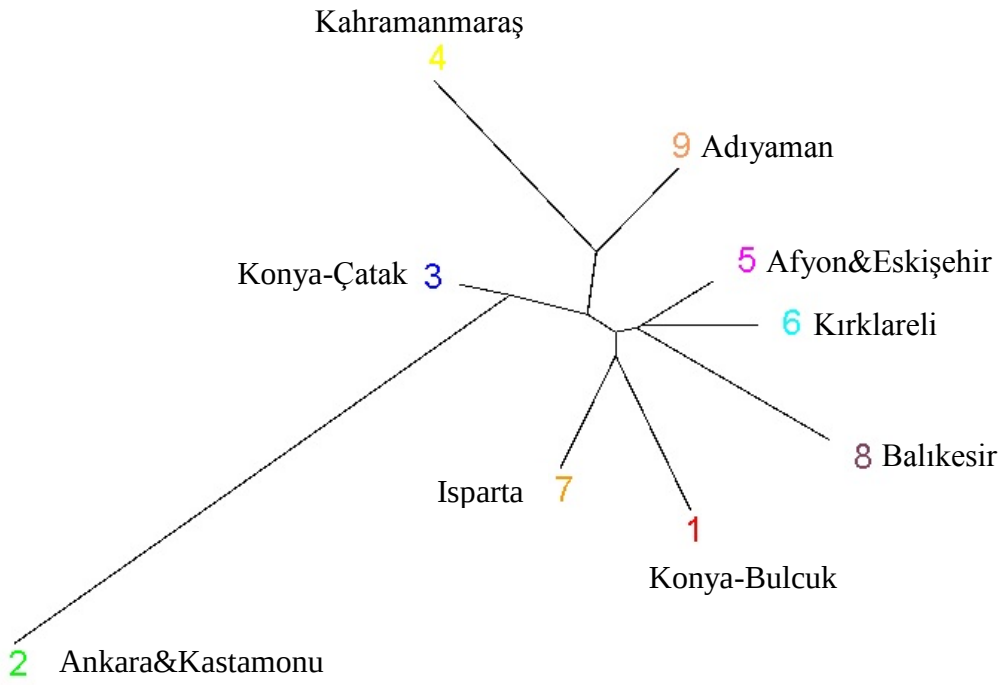


Figure 20 The constructed Neighbor-joining tree of Turkey oak samples for nine inferred clusters

In Figure 20, the Neighbor-joining tree of Turkey oak populations constituted according to the allele frequencies of 12 SSR loci was given. According to this result, nine genetically separated clusters were detected.

5.3 The Result of PCA Analysis without Suspected Hybrid Genotypes

Total 172 genotypes from 13 populations were analyzed. The results of PCA were given as two and three dimensional plots of analyses. The numbering codes for populations were given in Table 19.

Table 19 The list of 13 *Q. cerris* populations sampled from Turkey

Code	Populations
1	Kahramanmaraş- Boztoprak
2	Kahramanmaraş- Erince
3	Kahramanmaraş-Tırıl
4	Konya-Bulcuk
5	Konya-Çatak
6	Adıyaman
7	Isparta
8	Kırklareli- İğneada
9	Afyon & Eskişehir
10	Balıkesir- Dursunbey
11	Ankara-Çubuk
12	Kastamonu-Kayalı
13	Kastamonu-Küre

According to the results, 13 populations formed two distinct clusters. As one cluster included just Kastamonu and Ankara populations, the other had all the remaining populations sampled from various regions of Turkey (Figure 21A). The matrix correlation coefficient value was found r=0.70 which indicates a poor fit between distance-similarity matrix and similarity/dissimilarity dendrogram (Rohlf, 2008; Mohammadi and Prasanna, 2003). For three axes, a total of 44.68% variation was explained by Eigen values (Figure 21A). This big cluster was then examined in detail. The numbering codes for re-analyzed populations in the PCA were given in Table 20.

Table 20 The list of 10 *Q. cerris* populations and codes given in the PCA

Code	Populations
1	Kahramanmaraş- Boztoprak
2	Kahramanmaraş- Erince
3	Kahramanmaraş-Tırıl
4	Konya-Bulcuk
5	Konya-Çatak
6	Adıyaman
7	Isparta
8	Kırklareli- İğneada
9	Afyon & Eskişehir
10	Balıkesir- Dursunbey

Although a clear seperation did not occur among populations, it was observed that genotypes of spatially close populations tended to be located close (Figure 21B). According to the PCA of big cluster analysis, the first axis explained 29.80% of variation among genotypes and the cumulative value of Eigens was 44.51%. The correlation coefficient value was found $r<0.7$ indicating very poor fit (Rohlf, 2008; Mohammadi and Prasanna, 2003).

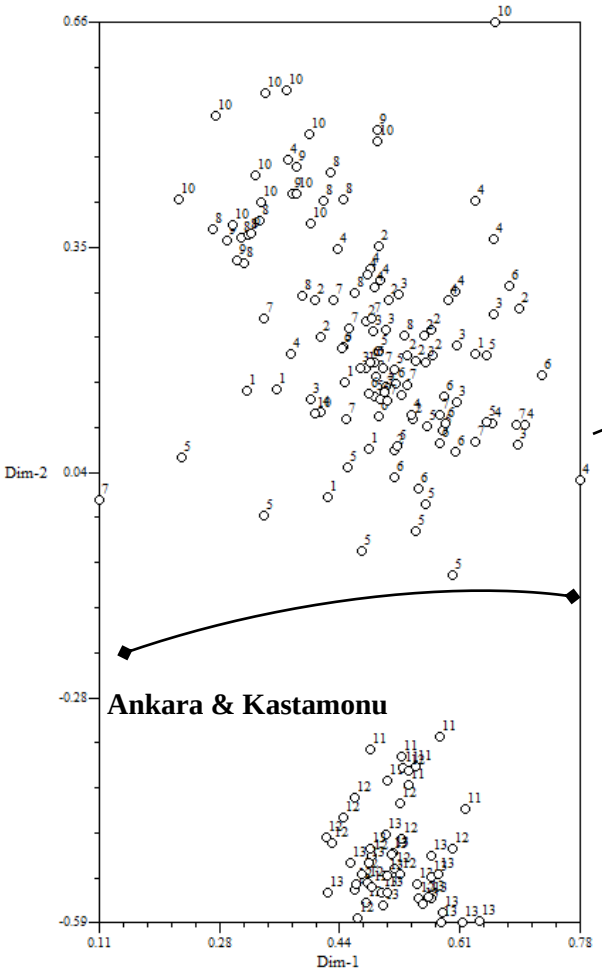


Figure 21A The two dimensional plot of Turkey oak populations

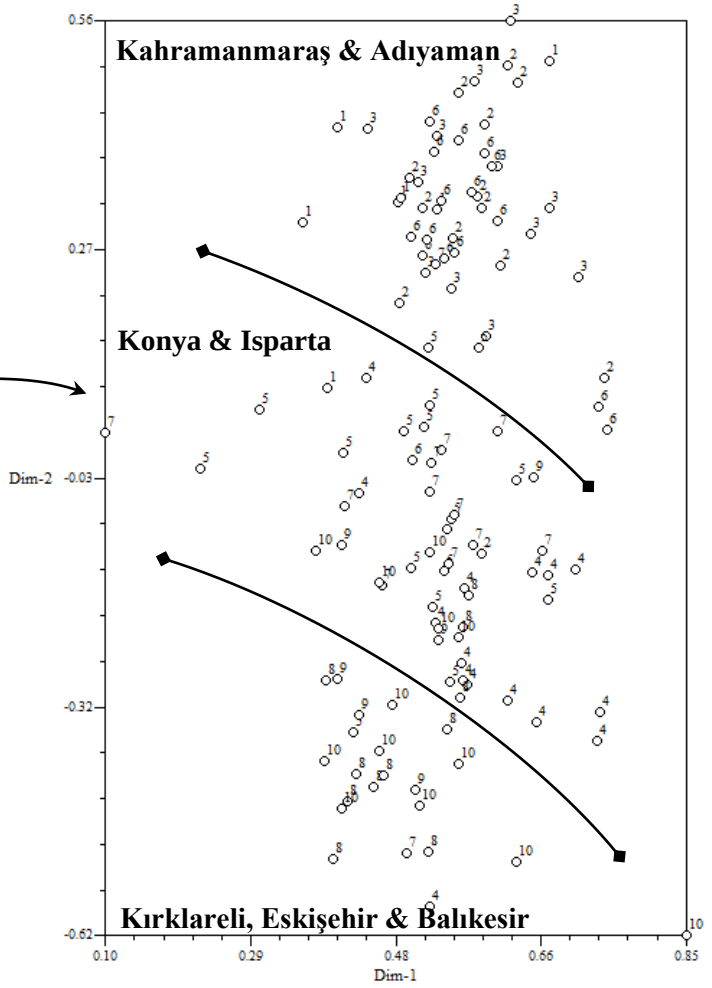


Figure 21B The focused 2-D PCA without northern Turkey populations

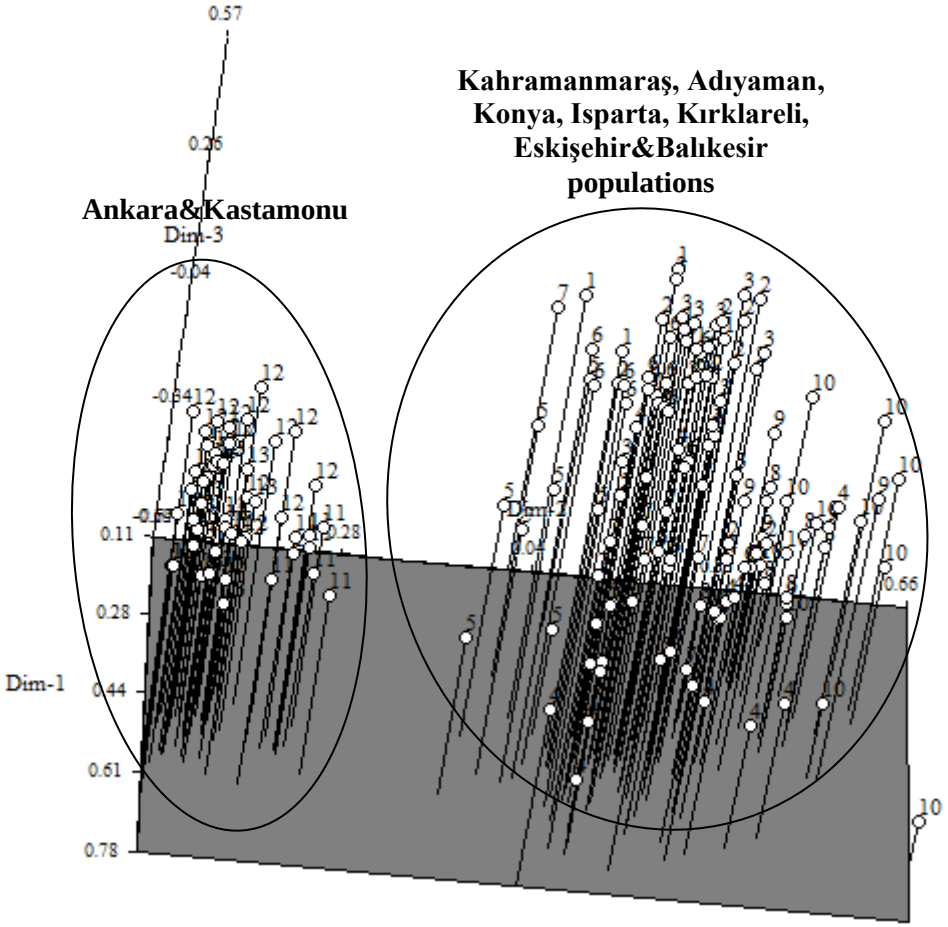


Figure 22A The three dimensional plot of Turkey oak populations

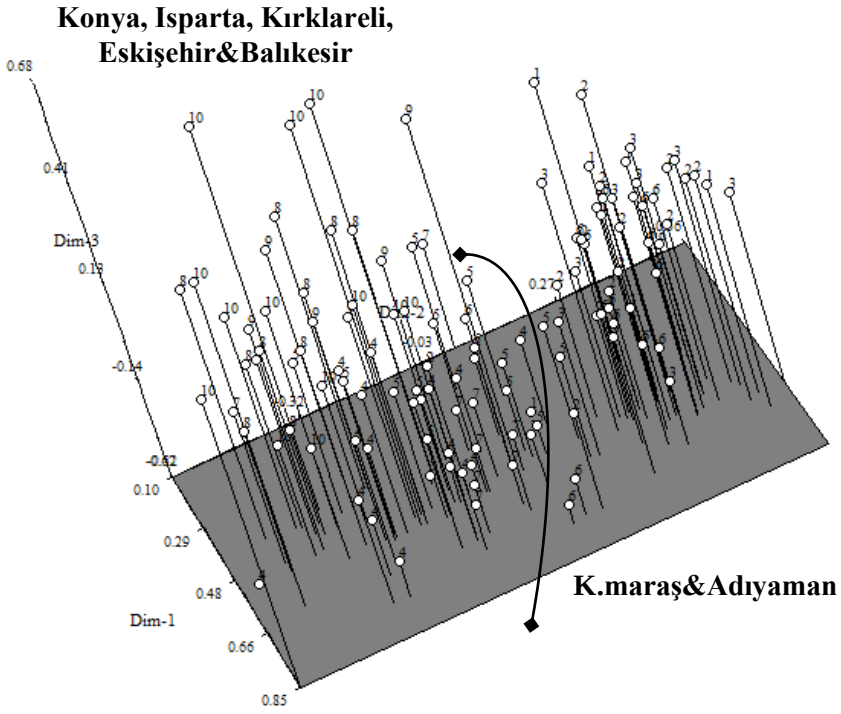


Figure 22B The focused 3-D PCA plot of Turkey oak populations

5.4 Regional PCA Analyses

5.4.1 Kahramanmaraş–Adıyaman Region PCA Analysis

Total 115 genotypes, 18 of which were suspected hybrids, were analyzed. 16 of these potentials were settled down in the group of *Q. cerris* and two of them were found in *Q. infectoria* group. The list of codes was given in Table 21. All suspected hybrid genotypes were shown as filled circles in two dimensional plots. Filled stars represented suspected hybrid genotypes having memberships for more than one population while unfilled ones used for regular genotypes but had memberships for more than one population (Figures 23A and 23B).

Table 21 The list of species included in Kahramanmaraş-Adıyaman Region analysis and codes given in the PCA

Code	Species
1	*Q.cerris*
2	*Q.cerris* var. *cerris* X *Q. infectoria*
3	*Q. infectoria*
4	*Q.cerris* var. *cerris* X *Q. brantii*
5	*Q. brantii*
6	*Q.cerris* var. *cerris* X *Q. libani*
7	*Q. libani*

According to the PCA results, the species of *Quercus* L. in the region were split up into two groups. One group included mostly *Q. infectoria* genotypes and the other had a mixture of *Q. cerris*, *Q. brantii* and *Q. libani* genotypes (Figure 23A). It was observed that *Q. cerris* genotypes were seperated from *Q. brantii* and *Q. libani*

genotypes in the secınd dimension. The similar formations were also seen in three dimensional plots (Figure 24A and 24B). The matrix correlation coefficient value was found to be *r*=0.75 (The data and related dendrogram were not given in thesis) which indicates a poor fit between distance-similarity matrix and similarity/dissimilarity dendrogram (Mohammadi and Prasanna, 2003; Rohlf, 2008). The eigen values calculated by NTSYS-pc for PC1, PC2 and PC3 were 27.06%, 8.04% and 5.14% respectively. Cumulative value of eigens in three axes was 40.26% (Figure 24A).

Table 22 The PCA list of species and the given codes

Code	Species
1	*Q.cerris*
2	*Q.cerris* var. *cerris* X *Q. brantii*
3	*Q. brantii*
4	*Q.cerris* var. *cerris* X *Q. libani*
5	*Q. libani*

In the detailed examination of *Q. cerris*, *Q. brantii* and *Q. libani* genotypes, three of suspected genotypes were grouped with *Q. cerris* and the remaining were close to *Q. brantii* and *Q. libani* genotypes (Figure 23B). According to the PCA results, the first axis explained 33.12% of total variation among genotypes and the cumulative value of Eigens was 44.73% (Figure 24B).

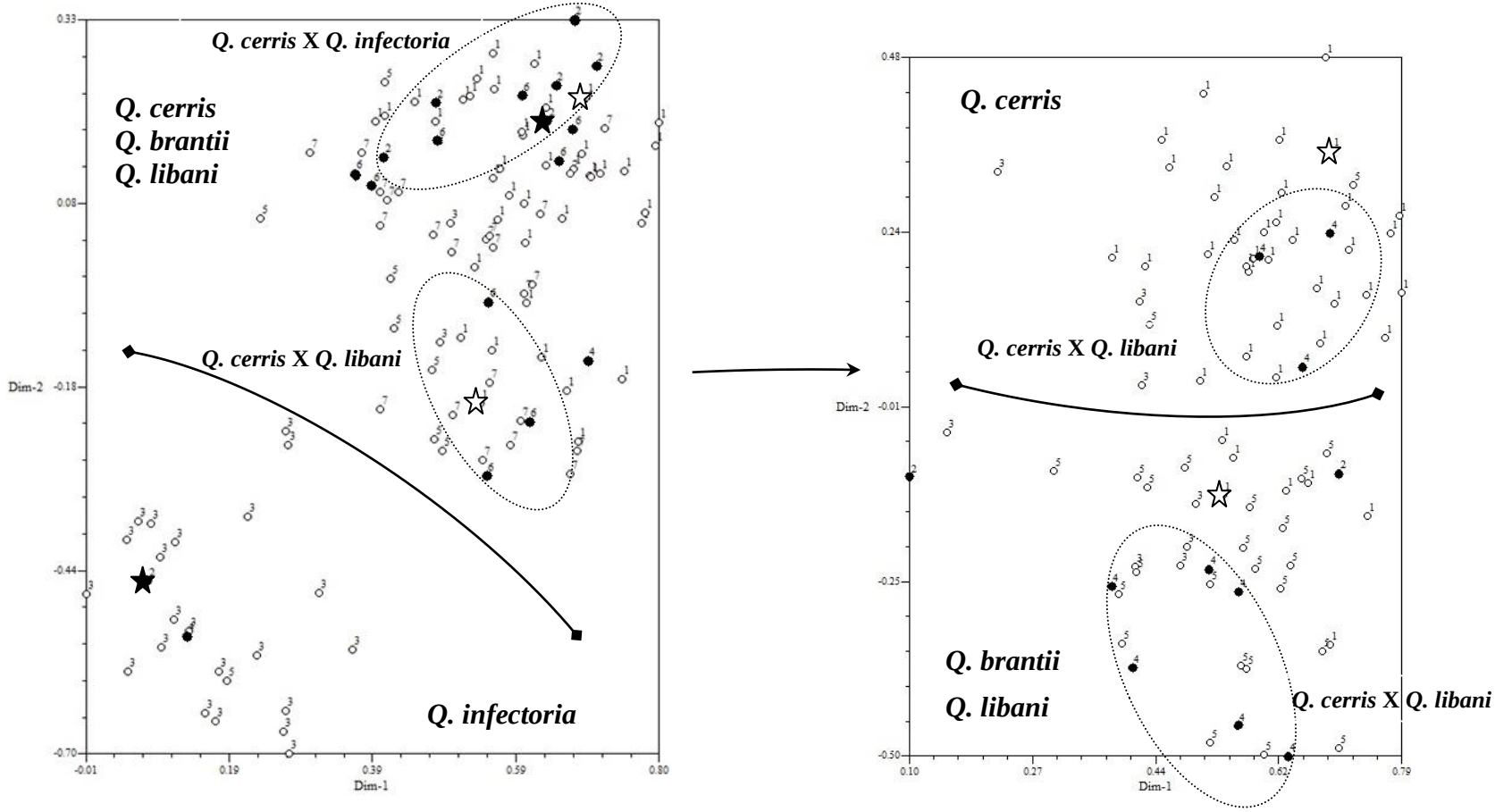


Figure 23A The 2-D plot of Kahramanmaraş-Adıyaman Region Figure 23B The PCA result that includes *Q. cerris*, *Q. brantii* and *Q. libani*

(○: Genotype attributed to a given species; ●: Suspected hybrid genotypes coming from different species combinations of oaks; ☆: Genotype attributed to a given species in this region but foundin Structure analysis having membership for the other region populations, too; ★: Suspected hybrid genotype and found in Structure analysishaving membership for the other region populations, too)

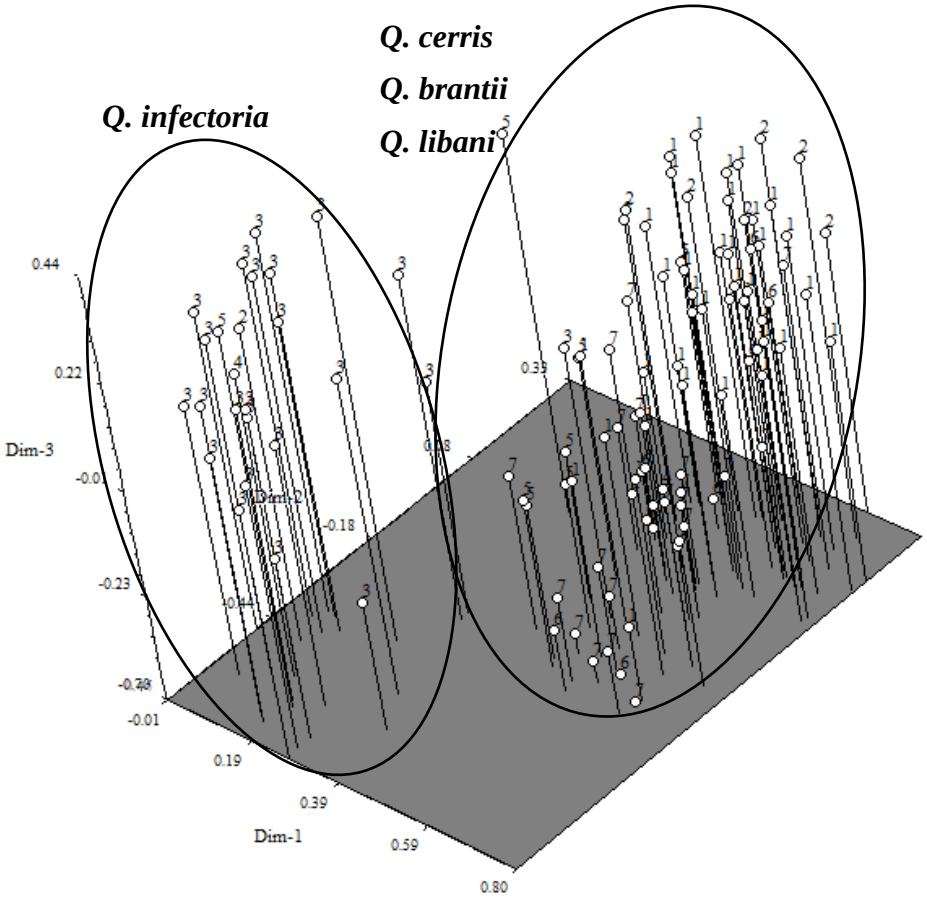


Figure 24A The 3-D plot of Kahramanmaraş-Adıyaman Region

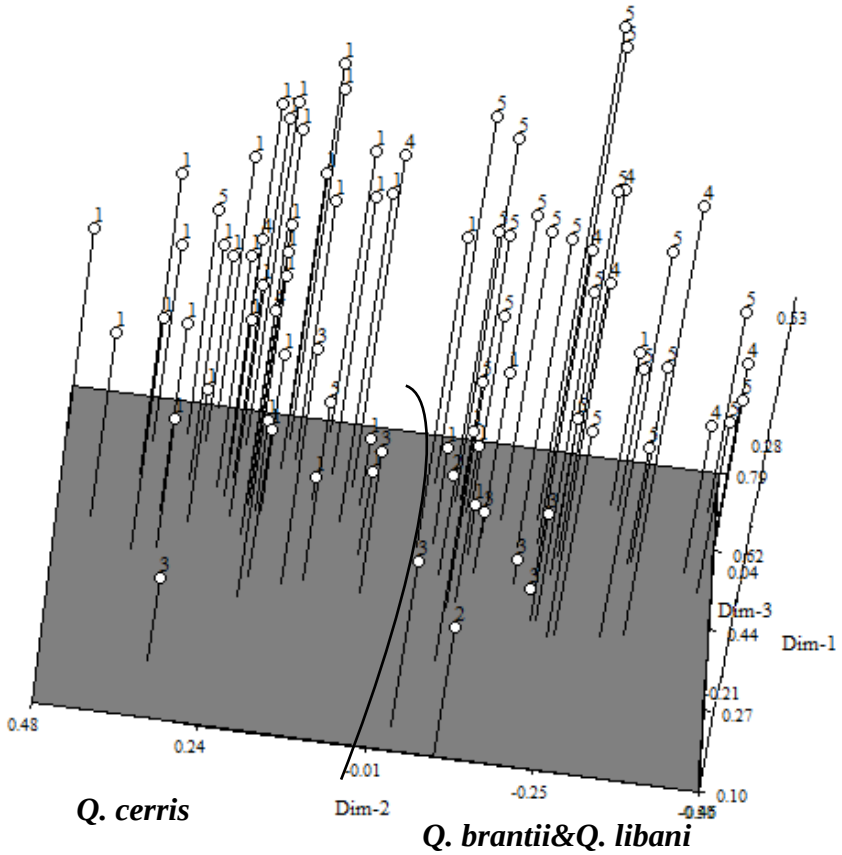


Figure 24B The focused PCA that includes *Q. cerris*, *Q. brantii* and *Q. libani*

5.4.2 Kastamonu-Ankara Region PCA Analysis

Total 97 genotypes which belong to three different species were analyzed in Kastamonu-Ankara region. Eleven of these genotypes were suspected hybrid genotypes. The result of PCA was given as two dimensional plot of the region's analysis. The list of codes was given in Table 23.

Table 23 The list of species included in Kastamonu-Ankara Region analysis and the codes given in the PCA

Code	Species
1	*Q.cerris*
2	*Q.cerris* var. *cerris* X *Q. infectoria*
3	*Q. infectoria*
4	*Q.cerris* var. *cerris* X *Q. pubescens*
5	*Q. pubescens*

The two dimensional plots of species in the Kastamonu-Ankara region were given in Figure 25. According to the results, the species were split up into two groups. One group included all *Q. cerris* genotypes and two different suspected hybrid genotypes (Figure 25). The other group had the rest of oak species and most of the suspected hybrid genotypes (Figure 25). In the assessment of this these species and their suspected hybrid genotypes, matrix correlation coefficient value was estimated r=0.91 (The data and related dendrogram were not given in thesis). This indicates a high correlation between distance-similarity matrix and similarity/dissimilarity dendrogram (Mohammadi and Prasanna, 2003; Rohlf, 2008). According to the Eigen values, the first component explained 26.87% of variation among genotypes. The total variation explained by three components was calculated 49.79%.

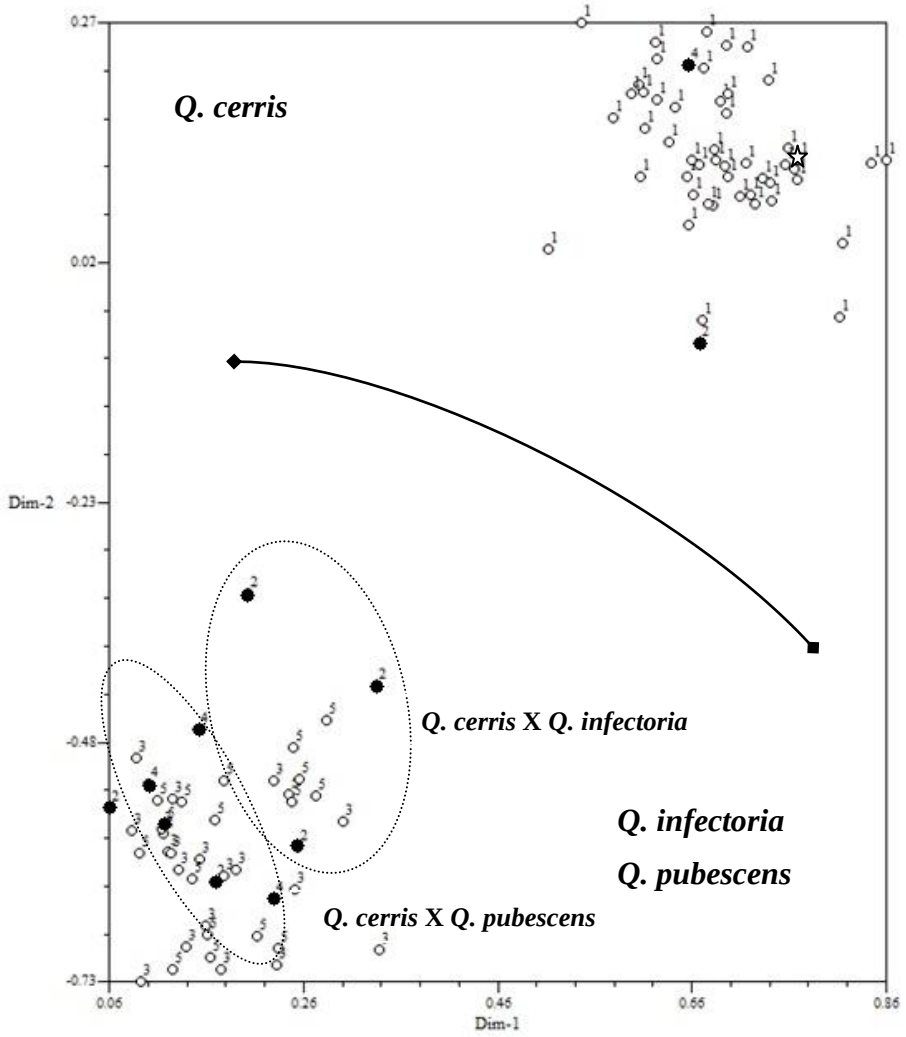


Figure 25 The 2-D plot of Kastamonu-Ankara Region

(○: Genotype attributed to a given species; ●: Suspected hybrid genotypes coming from different species combinations of oaks; ☆: Genotype attributed to a given species in this region but foundin Structure analysis having membership for the other region populations, too; ★: Suspected hybrid genotype and found in Structure analysishaving membership for the other region populations, too)

5.4.3 Konya-Isparta Region PCA Analysis

Total 119 genotypes which belong to four different oak species were analyzed in Konya-Isparta region. Six of these genotypes were suspected hybrid genotypes. The result of PCA was given as two dimensional plot of the region's analyses. The list of

codes for the species was given in Table 24 and all suspected hybrid genotypes were shown with filled circles in 2-D plot (Figure 26).

Table 24 The list of species included in Konya-Isparta Region analysis and the codes given in the PCA

Code	Species
1	*Q. pubescens*
2	*Q.cerris* var. *cerris* X *Q. pubescens*
3	*Q.cerris*
4	*Q.cerris* var. *cerris* X *Q. infectoria*
5	*Q. infectoria*
6	*Q.cerris* var. *cerris* X *Q. trojana*
7	*Q. trojana*

The two dimensional plots of regional analyses were given in Figure 26. According to the results, studied oak species were split up into two groups. One group included *Q. infectoria* and *Q. pubescens* genotypes and three different suspected hybrid genotypes. The other group had *Q. cerris* and *Q. trojana* genotypes and three suspected hybrid genotypes. The similar formation was also observed in three dimensional plots, too (The data was not given in thesis). Matrix correlation coefficient value was found to be r=0.81 for this region's populations. This value is the representative of good fit (Mohammadi and Prasanna, 2003; Rohlf, 2008). According to the Eigen values, the first component explained 17.02% of variation between genotypes. The cumulative value of total variation explained by three components was foundto be 37.36%.

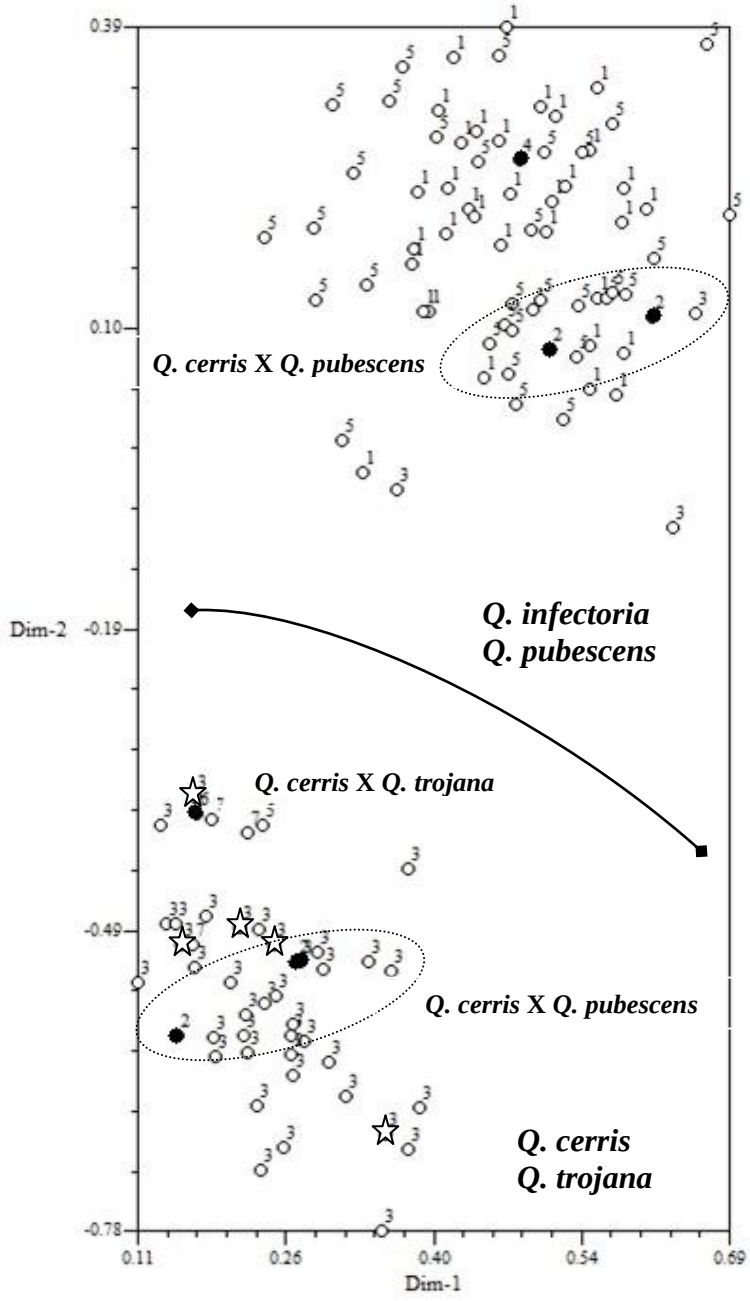


Figure 26 The 2-D plot of Konya-Isparta Region

(○:Genotype attributed to a given species; ● : Suspected hybrid genotypes coming from different species combinations of oaks; ☆: Genotype attributed to a given species in this region but foundin Structure analysis having membership for the other region populations, too; ★ : Suspected hybrid genotype and found in Structure analysishaving membership for the other region populations, too)

CHAPTER 6

DISCUSSION

6.1 Loci-based Genetic Diversity and Diversity Pattern in Turkey Oak

In the study of Steinkellner et al. (1997b), the activity of a primer other than the target species was discussed and designated to evolutionary and genetic distance of species. It was stated that when the genetic distance increased among species, the cross species amplification success of primers decreased (Steinkellner et al., 1997b; Barreneche et al., 1998). The primers ssrQpZAG9 and ssrQpZAG36 which were tested in that study were also used in the present study. They detected these primers were polymorphic for *Q. cerris* samples. The primers were also found polymorphic in the current study. These loci were originally designed for *Q. petraea* which is a member of the same subgenus with *Q. cerris*. The highest private allele number was detected in ssrQpZAG9 loci in this study. This is a good indicator of allelic diversity in terms of the studied genotypes. Also, the highest observed heterozygosity value was found to be 0.70 for the loci ssrQpZAG9 and 0.65 for ssrQpZAG36. Negative results of *Fis* of these loci means excess of heterozygotes, too. So, the application of these primers to *Q. cerris* genotypes were successful and are offered for the future studies.

Among the tested SSR primers, four microsatellites were originally designed for *Q. mongolica* var. *crispula*. This species is another member of the subgenus *Quercus*. As far as is known, these primers were tested in *Q. cerris* for the first time. According to the A and Ap results, the microsatellite QmC00932 had the highest values indicating allelic richness. The private allele numbers of these primers

decreased with addition of suspected hybrid genotypes to the analysis. The only expection was the locus QmC00932. The private allele number of this microsatellite increased with suspected hybrid genotypes. This means that the locus have a high selectivitiy power and the allelic structure of new genotypes differs only for this loci. The loci which had negative *Fis* values were QmC01758 and QmC00716. This indicates excess of heterozygotes. The loci QmC00932 and QmC00963 were favoring homozygosity with positive values. Also *Fst* values of QmC01758 and QmC00716 were higher than QmC00932 and QmC00963 indicating higher contribution to genetic differentiation of populations. For these reasons, the loci QmC01758 and QmC00932 are more preferrable than the other two for the future studies.

Five of the SSR primers were originally developed for *Q. acuta* which is a member of the subgenus *Cyclobalanopsis*. As far as is known, these primers were tested in *Q. cerris* for the first time, too. According to descriptive statistics results, the lowest allelic diversity in terms of allele numbers and observed heterozygosity was observed for the loci MSQa8 favoring the lowest genetic diversity. The higher positive *Fis* value of this loci MSQa8 means excess of homozygotes, too. However, with the highest *Fst* value, this locus' contribution to genetic differentiation of Turkey oak populations was the highest of all primers. So, it may be stated that the leastinformative locus was MSQa8 for the genetic diversity analysis of Turkey oak samples. Only two *Q. acuta* loci, MSQa3 and MSQa11, had negative *Fis*, higher *Fst* values and at moderate level of A and Ap values. They may be applied for Turkey oak genotypes and used in the future studies. Although these five primers were proposed transferrible to the other three species of *Cyclobalanopsis* (Lee et al., 2010), these findings support the statement that increased genetic distance decrases the amplification success of primers. In general, the genetic application and the transferrability of MSQa8, MSQa10 and MSQa14 to *Q. cerris* is not proposed for the future studies.

In terms of the studied SSR loci genetic diversity and *F* statistics results, addition of suspected hybrid genotypes to analysis decreased private allele numbers, mean number of alleles per locus and per polymorphic locus. So, the selectivitiy power of

primers decreased. Negative *Fis* values of loci decreased from seven to three meaning a general increase in homozygosity loci. This is the result of new alleles and the allelic structure of new genotypes. The mean *Fst* value for all populations and for all loci decreased with additional genotypes implying decreased genetic differentiation among populations.

6.2 Population-based Genetic Diversity and Diversity Pattern in Turkey Oak

6.2.1 Discussion without Suspected Hybrid Genotypes

Genetic Diversity Parameters and *F* Statistics

According to the results of genetic diversity analysis by excluding suspected hybrid genotypes, South-Central Turkey populations had the highest private allele number in total. Southeastern Turkey populations' private alleles were also high. This is a good indicator of allelic diversity in terms of the studied genotypes and microsatellites. As well as the least allelic diversities and also the lowest proportion of polymorphic loci values and mean number of alleles were observed in Ankara and Kastamonu populations. The reason to this can be explained by nonrandom mating in these populations and low cross-pollination. Individual populational *Fis* values and the mean value of this parameter for Northern Turkey populations support this idea. This is also the reason to the highest fixation index of this region in comparison to the Southeastern and South-Central Turkey populations. The genetic uniformity of subpopulations causes increased genetic differentiation among populations. Four populations which were the Kahramanmaraş-Boztoprak and Tırıl, Konya-Bulcuk and Isparta populations had negative values of *Fis* favoring heterozygosity. This may be due to efficient gene flow and randomly mating in each of these populations. In general, it can be stated thatKahramanmaraş-Adıyaman and Konya-Isparta regions were the most genetically diverse regions. The lowest genetic differentiation among populations was found in Kahramanmaraş-Adıyaman region populations and than in South-Central Turkey populations. Besides this, populations that had the least genetical diversity were from Kastamonu and Ankara regions. This results were the

expected ones in accordance to populations' structures. Populations from Kahramanmaraş-Adıyaman and Konya-Isparta regions were rich in *Quercus* species. However, populations from Kastamonu and Ankara were less diverse in terms of oak species. This indicates the role of hybridization and the tendency of genetic mixture in the regions where the oak species are abundant and had overlapping distribution such as Konya-Isparta and Kahramanmaraş-Adıyaman regions.

Genetic Structures of Populations and Genetic Distance among Them

According to dendrogram, two major groups were observed. One group included Ankara-Kastamonu zone populations and the other larger one had all the remaining populations sampled from Anatolian peninsula and Thrace. For the smaller cluster of Ankara-Kastamonu, Kayalı and Küre populations of Kastamonu formed a group to which Ankara-Çubuk population joined. The larger cluster was split into two groups, too. One of them included Kahramanmaraş-Adıyaman populations. In this cluster, it was observed that Erince and Boztoprak populations were genetically more closer than Tırıl population of the same region. The other had Konya, Isparta, Kırklareli, Eskişehir and Balıkesir populations. In general, it was observed that geographically close populations were found genetically close, too. Also the dendrogram result was compatible with statistical analysis results. The genetic distance between two major groups was found remarkably high. It can be stated that Kastamonu and Ankara populations are the most seperated populations both spatially and genetically. On the contrary, Kahramanmaraş and Konya-Isparta populations internally were the genetically closest populations due to the distance matrix results. This supports the low inbreeding and genetic divergence results of these regions.

The results of population structure analysis revealed two probable and genetically homogeneous groups. This result is also compatible with genetic distance and statistical analysis results. According to the quantitative clustering method that the Structure program performs, nearly allgenotypes originated from either of two probable inferred populations with more than 0.90 membership of coefficients. Just two genotypes had memberships in bothclusters. The coefficients of these genotypes

were 0.61 and 0.39 for the clusters Ankara-Kastamonu and the larger cluster that had the rest of the populations, respectively. These two genotypes were from Balıkesir population. Unlike the other members of the Balıkesir population, they were inferred genetically closer to Kastamonu-Ankara cluster.

Most of the samples analyzed in this thesis studies were *Q. cerris* var. *cerris*. Among 172 genotypes, just 11 genotypes were *Q. cerris* var. *austriaca*. Kırklareli-İğneada population which included the representatives of this variety were grouped into the cluster covering west central Anatolian till Thrace. As for the Stucture analysis, the variety *Q. cerris* var. *austriaca* was defined genetically close to all populations from different regions except Kastamonu and Ankara. With respect to these statistical and structural results, there was no distinctive difference among these two variety of Turkey oak species. However to do a beter understanding, there should be more genotypes included into the analysis within a more comprehensive study.

In the assessment of 13 *Q. cerris* populations sampled from different regions of Turkey without suspected hybrid genotypes, populations formed two distinct clusters in PCA analysis. While Ankara and Kastamonu populations grouped in one clump, all the remaining populations formed second cluster. Although the correlation coefficient value was found to be low, the results of PCA could not be interpretedas insignificant (Mohammadi and Prasanna, 2003). Moreover, the results of two and three dimensional plots were found to be quite compatible with UPGMA dendrogram and the results of Structure analysis.

6.2.2 Discussion with Suspected Hybrid Genotypes

Genetic Diversity Parameters and *F* Statistics

According to the statistical results of genetic analysis with suspected hybrid genotypes, Kastamonu-Küre and Ankara-Çubuk populations had the lowest proportion of polymorphic loci values. Besides this, the least allelic diversity was

found in these populations. These results indicate that this region's populations show the lowest genetic diversity than the others. The reason to this can be inbreeding which distributes genetic content from heterozygous to homozygous state. Positive *Fis* values of these populations supports this idea. On the contrary, the Southeastern and South-Central Turkey populations were rich in private alleles and their mean number of alleles per locus and per polymorphic locus were found higher than the Northern Turkey populations. Lower inbreeding coefficients within subpopulations of these region's populations indicate efficient gene flow and excess of heterozygotes. However, there was a common tendency favoring inbreeding in terms of all populations. It can be emphasized that Kahramanmaraş-Adıyaman and Konya-Isparta regions were the most diverse regions. Most of the variation at genetical level was found in these regions. Populations from Kahramanmaraş-Adıyaman and Konya-Isparta regions were rich in *Quercus* species belonging to section *Cerris* and they did not form pure stands. However, populations from Kastamonu and Ankara were mixed mostly with the species from section *Quercus* with which *Q. cerris* makes rare hybridizations.

When the results of analysis with or without suspected hybrid genotypes were compared, the general statements did not change at all. For example, in both analyses Ankara-Kastamonu region's populations had the lowest values of population genetic parameters and highest inbreeding results. On the contrary, populations from Southern Turkey had the highest allelic as well as genetic diversity at all. South-Central Turkey populations gave similar results to Southeastern Turkey populations in both analysis, too. One significant difference was the increase in allelic diversity for Kahramanmaraş-Erince population. By the addition of suspected hybrid genotypes into the analysis, significant change was observed in the A and Ap values. All the other populations to which suspected hybrid genotypes added, A and Ap values were also increased. At this point, extra genotypes' number were not same for all populations. The highest number of extra genotypes were added to the populations Adıyaman, Kastamonu-Kayalı and Kahramanmaraş-Erince as 10, 10 and 16, respectively. To the other populations, 1, 2 or maximum 4 samples were added. Higher increase in allelic diversity at more extra genotype added populations than the others is reasonable due to the greater number of potantial alleles may be found in

more individuals. This result was definitely the cause of additional genotypes with potential hybridity to these populations although does not necessarily mean that these genotypes are hybrid individuals. The populations without any suspected hybrid genotypes were the Kastamonu-Küre, Kahramanmaraş-Boz and Tırıl and Kırklareli-İğneada. As expected, no changes occured in estimated population genetic parameters of these populations.

When the results of *F* Statistics were compared, it was observed that there was an increased inbreeding at all populations and regions except the ones did not have extra genotypes. Same as inbreeding values, *Fit* and *Fst* values were also increased. However, it might be expected an increased heterozygosity by the addition of extra genotypes owing to their allelic diversity. There may be several explanations of this situation. According to the results of several hybrid detection studies in oak species, it has been stated that although exhibiting high morphological intermediacy and having tendecy to interspecific hybridization, the most of these individuals may not be necessarily hybrids (Curtu et al., 2007; Conte et al., 2007). Our suspected genotypes may not be hybrids, too. In such a circumstance, these genotypes would maintain their genetic identity same as with one of its suspected parental species. So, this would not be related with the inbreeding tendency of those populations. Another explanation may be related with the hybrid's inviability and infertility, favoring backcrossing to a particular parent and genomic barriers acting against hybrids maintaining species specific identities (Rushton, 1993; Lexer et al., 2006; Curtu et al., 2007; Conte et al., 2007). All of these are the result of interspecific gene flow occurs in oak species. In such a circumstance, our suspected genotypes may be introgressed forms backcrossed to one potential parent. A more parent like individual with an enriched genetic material due to hybridization will occur. This may explain increased allelic diversity. To better understand hybridity between *Q. cerris* and other *Quercus* species, an exact hybrid detection survey should be done. Such kind of a study will especially be valuable when it is considered that Turkey is an oak species rich country.

Population Structure and Genetic Relationship among Populations

Two major clusters were observed in the dendrogram of Turkey oak populations with suspected hybrid genotypes. The smallest cluster was constituted by Ankara-Kastamonu populations as in the analysis without suspected hybrid genotypes. The Kastamonu populations were grouped together to which Çubuk population was joined. The larger cluster was split into two clusters same as in the analysis without suspected hybrid genotypes. One group included Kahramanmaraş-Adıyaman populations. Rather than the Erince and Boztoprak populations, Boztoprak and Tırıl populations were found to be genetically closer to each other in this group. This was clearly the effect of suspected hybrid genotypes added to the Erince population in Kahramanmaraş region. The other group in the larger cluster included Konya, Isparta, Kırklareli, Eskişehir and Balıkesir populations. Suspected hybrid genotypes had also effects on this cluster, too. The Çatak population was found to be closer to the Bulcuk population of Konya and Isparta population. The least correlated population to this cluster was the Balıkesir-Dursunbey as expected due to geographical distance. The genetic distance between two major clusters was found remarkably high. Same as the analysis without suspected hybrid genotypes, the Kastamonu-Ankara was the most seperated populations both spatially and genetically. The highest genetic differentiation found for these region's populations also supports dendrogram results.

According to the results of structural analysis of populations with extra suspected hybrid genotypes, the best inferred number of populations was nine. However, the defined population number was 13. So, some of the populations were genetically inferred that they belong to the same clusters. For example, three Kahramanmaraş populations were clustered as one population. Similarly, Ankara-Çubuk and two Kastamonu populations were genetically inferred in the same cluster. This was a predictable circumstance due to geographical proximity of these populations. If NJ and UPGMA trees were compared in terms of structural organization of populations, it can be said that there was no significant difference among them. However, one interesting thing was the genetical proximity of Konya-Çatak population to Ankara

and Kastamonu populations. That population was defined close to Konya-Isparta populations in UPGMA analysis of suspected hybrid genotypes included analysis. Surprisingly, in the analysis without suspected hybrid genotypes, although being in the same cluster, Çatak was the least related population to its group. When the genotypes were evaluated according to the membership of coefficients found by the structural analysis of populations, it was observed that a few genotypes had memberships in different clusters which were not defined as suspected hybrid genotypes during the field study. These genotypes' genomic structure were computed belonged to more than one populations. For example, a genotype from Kastamonu-Küre had memberships to the Kastamonu-Ankara cluster and the Kahramanmaraş-Adıyaman cluster. This may be the result of microsatellite data and increased primer application to increased number of genotypes may change the results.

When the results with 219 genotypes were considered with regard to *Q. cerris* var. *austriaca*, there was no significant difference between two varieties. Such as in the analysis without suspected hybrid genotypes, Kırklareli-İğneada population grouped into the cluster covering the area from west central Anatolia to the Thrace. The Structure analysis' results changed with addition of suspected hybrid genotypes. According to the dendrogram, the Kırklareli population was found to be closer to Afyon, Eskişehir and Balıkesir populations than to rest. For a better understanding of the relationship between two varieties, more samples with more SSR primers should be conducted in the future studies.

6.3 Genetic Relationship between *Quercus cerris* L. and the Other Codistributed Oak Species in the Regional Content

6.3.1 Kahramanmaraş-Adıyaman Region

In this region's analysis, four different oak species and three suspected hybrid genotype kinds were analysed. According to the PCA results, these species were found to split up into two groups. One group included *Q. infectoria* members as the other had *Q. cerris, Q. brantii* and *Q. libani* samples. This seperation is compatible

with the seperation of genus *Quercus* members as sections in Turkey previously defined by Davis (1971) and Hedge and Yaltırık (1982). The species were clustered as section *Quercus* (*Q. infectoria*) and section *Cerris* (*Q. cerris*, *Q. brantii* and *Q. libani)*. When the data was reanalyzed by excluding *Q. infectoria* genotypes and its potential hybrid genotypes, the detailed information on section *Cerris* members was obtained. This time, seperation between species were not as clear as the previous one. However, the accumulation of *Q. cerris* genotypes to one side and the other two species *Q. brantii* and *Q. libani* in the other side of the plot was observed. This means that *Q. cerris* still maintaining entity in spite of coexistence with other related species.

When 18 suspected hybrid genotypes were comparatively diagnosed according to PCA and Structure programme results, it was observed that just two of the suspected hybrid genotypes had membership for more than one populations. Those genotypes were indicated as filled stars. The one which was located in section *Quercus* with *Q. infectoria* members was originally a member of Kahramanmaraş-Erince population. It was found to have membership of coefficient; 0.31 for Konya-Çatak population, 0.24 for Kahramanmaraş-Adıyaman zone populations and 0.37 for Isparta population. This means that the genetic composition of this individual is admixed in terms of 12 microsatellite loci. The finding of the highest coefficient of membership for Isparta population corresponds to region that has the highest *Q. infectoria* spread. The other genetically admixed sample was a member of Adıyaman population and found in section *Cerris* in PCA. It was calculated to have memberships 0.30 and 0.63 for Balıkesir and Adıyaman populations in respect. Remaining 16 suspected samples were not admixed genetically. They were all located within section *Cerris* and had membership for their defined populations with more than 0.94 coefficients.

Other than these suspected genotypes, two samples were found to have membership in more than one population. They were marked in the figures as unfilled stars. They were *Q. cerris* genotypes and located in section *Cerris* cluster. The inferred clusters were Çatak, Isparta, Kahramanmaraş and Kırklareli populations. In the detailed two dimensional plot, these genotypes were seperated and located near *Q. brantii*-*Q. libani* or *Q. cerris*. The genotype that observed next to *Q. cerris* samples computed

to have memberships 0.49% and 0.29% for Kahramanmaraş and Kırklareli populations. The other one was found genetically similar to Çatak, Kahramanmaraş and Isparta populations with 0.18, 0.48 and 0.31 membership of coefficients. It was located near *Q. brantii-Q. libani* samples' side.

6.3.2 Kastamonu-Ankara Region

In Kastamonu-Ankara region, three different species and two suspected hybrid genotype kinds were analysed. According to the PCA results, members of species formed two distinct clusters. One cluster included *Q. cerris* genotypes and the other had *Q. pubescens, Q. infectoria*. This clustering is compatible with species' membership to Turkey *Quercus* species' sectional design. As members of section *Quercus* locating together, *Q. cerris* samples formed a different cluster.

When suspected genotypes were comparatively diagnosed with the PCA analysis, most of the suspected hybrid genotypes were located in the cluster including *Q. pubescens* and *Q. infectoria*. According to the Structure programme results, it was observed that all samples belong to their predefined population (Kastamonu-Ankara) with a high genomic proportion (>0.84). This means that the genetic composition of suspected genotypes were not genetically admixed.

Other than these suspected hybrid genotypes, one sample was detected to have membership in more than one population. It was also marked as a unfilled star. It was *Q. cerris* sample and located in section *Cerris* cluster. The inferred clusters were Ankara-Kastamonu populations and Kahramanmaraş region populations with the coefficients 0.54 and 0.43, respectively. This means that this sample genetically similar to Ankara-Kastamonu populations in 0.54% and to Kahramanmaraş region populations 0.43%.

6.3.3 Konya-Isparta Region

In the Konya-Isparta region, four different oak species and three suspected hybrid genotype kinds were analysed. According to the results, members of the same section formed a clear seperate cluster. The species *Q. infectoria* and *Q. pubescens* which belong to section *Quercus* formed one group and *Q. cerris* and *Q. trojana* that are members of section *Cerris* formed the other group.

Total six suspected hybrid genotypes were comparatively diagnosed with PCA and Structure programme. It was observed that all these genotypes belong to their inferred ancestry (Konya-Isparta) with a high membership value (>0.95). This means that the genetic composition of suspected genotypes were not genetically admixed.

Other than these suspected genotypes, five samples were analyzed to have memberships in more than one population. They were also marked as unfilled stars. All of them were *Q. cerris* genotypes and located in section *Cerris* cluster. They had at least two and maximum three ancestries computed by the Structure programme. When the highest membership of coefficients were taken into the account, it was seen that three inferred ancestries belonged to three populations of Isparta, Çatak and Adıyaman with membership of coefficients of 0.36, 0.42, 0.62, respectively. This means that genetic compositon of these genotypes are genetically admixed in terms of 12 microsatellites.

CHAPTER 7

CONCLUSION

The main objective of this study was to determine the magnitude and pattern of genetic diversity of Turkey oak populations sampled from its natural range of Turkey. Additionally, it was to investigate the role of hybridization among closely related species of Turkey oak in hybrid regions on genetic structure of Turkey oak populations.

To meet the objectives of the study, SSR marker system was chosen since it is a commonly used, accurate and valuable analysis technique due to its codominant nature. Although none of them originally developed for Turkey oak, all microsatellite loci developed for other oak species that were used in the study were polymorphic for *Q. cerris*. However, primers designed for species which are members of the same subgenus with Turkey oak gave better results.

Estimated population genetic parameters of the study, Turkey populations indicated that Kahramanmaraş-Adıyaman hybrid region populations had the highest genetic diversity and lowest genetic differentiation of populations within the region due to efficient gene flow resulting in excess of heterozygotes. Similar genetic diversity pattern was also observed in the Konya-Isparta oak rich region. The oak species richness of these regions belonging to the same section as *Q. cerris*, all coexisting and probably hybridizing, also supports the results. The Kahramanmaraş-Adıyaman and Konya-Isparta oak rich regions could be considered as diversity center of Turkey oak. The gradual decreasing of genetic diversity from Southeastern to South-Central and distinctive decline from Southern to Northern Turkey populations may be related with paleobotanical distribution of the species and the effects of refuge zones from

have occured in Turkey oak. The interruption between North and South may be due to antropogenic effect substantially observed around the country.

Grouping of the Turkey oak populations based on genetic distances among them revealed two major clusters. One of them included all Southeastern and South-Central Turkey populations while the other cluster consisted of the Northern populations (Kastamonu-Ankara). This pattern does not change much with addition of suspected hybrid genotypes to the analyses. When the most probable number of populations (*K*) was estimated, two probable and genetically homogenous groups were again detected. However, there were nine clusters when suspected hybrid genotypes were added to the analysis. For nine clusters, Kahramanmaraş-Adıyaman populations and Kastamonu-Ankara populations formed large groups as the rest formed their own clusters. These results supported that there is a genetic diversity gradient from east to west in the Southern Turkey and disrupted northern populations of Turkey oak.

The differences between both types of sampling material (with and without suspected hybrid genotypes) were the overall increase in population genetic parameters. The mean values of proportion of polymorphic loci as well as average number of alleles per locus and per polymorphic locus increased by inclusion of suspected hybrid genotypes. As the mean expected heterozygosity increased with the addition of suspected hybrid genotypes, mean observed heterozygosity was decreased. Although the opposite was expected as an effect of suspected hybrid genotypes, overall tendency to inbreeding and genetic differentiation among populations of regions was increased. This contradictory results may be related with the sterility of hybrid individuals and possible removal of them due to several reasons such as selective cutting of the sterile hybrids by local people since sterile hybrids do not produce acorns. Thus, the maintenance of genetic identity of Turkey oak species in studied areas is commonly correlated with genomic barriers which prevent putative hybrid production.

The information on magnitude and pattern of genetic diversity in Turkey oak obtained for the first time with the current study will be useful for establishing principles for conservation and management of genetic resources of the species. Currently, Forest Tree Seeds and Tree Breeding Research Institute, Ministry of Forestry and Water Affairs sets up seed stands and gene conservation forests for economically important tree species including Turkey oak without genetic data. By considering the genetic information yielded by the current study, effective conservation and management programs should be implemented for Turkey oak since most of Turkey oak stands are located in highly degraded oak forests in Turkey.

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APPENDIX A

An Example File for GDA Programme

```
#nexus
[!Data from Quercus
]
begin GDAdata;
dimensions nloci=12 npops=13;
format tokens missing=? datapoint=standard;
locusallelelabels
1   'P5' [/ 1 2 3 4 5],
2   'P39' [/ 1 2 3 4 5 6 7 8 9 10 11 12],
3   'P9' [/ 1 2 3 4 5 6 7 8 9 10 11 12],
4   'P3' [/ 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15];

matrix
ERC:
17 ERC      1/4     1/8     1/9     8/10    3/3     3/10    5/5     1/12    2/2
26 ERC      1/1     1/8     1/9     6/9     7/7     12/14   4/4     1/12    ?/?
28 ERC      ?/?     ?/?     ?/?     ?/?     ?/?     6/8     5/5     1/12    ?/?
BOZ:
76 BOZ      1/4     1/8     1/9     6/6     ?/?     ?/?     ?/?     ?/?     2/2
77 BOZ      1/1     1/8     ?/?     6/7     6/6     6/10    5/5     1/12    2/2
78 BOZ      1/4     2/11    1/9     6/7     ?/?     ?/?     ?/?     ?/?     2/2
80 BOZ      1/4     2/2     1/9     6/6     ?/?     ?/?     ?/?     ?/?     2/2
TRL:
128KAH      1/4     2/10    8/9     6/7     3/3     3/8     5/5     1/11    ?/?
133KAH      1/4     2/10    1/9     6/11    3/3     5/8     4/4     13/13   2/2
135KAH      1/4     2/2     1/9     6/11    3/6     5/14    1/2     1/12    ?/?
136KAH      1/4     2/10    1/9     6/7     3/11    3/10    ?/?     1/12    2/2
```

APPENDIX B

An Example File for STRUCTURE Programme

Genotypes	Primers							
	p5	**p39**	**p9**	**p3**	**p33**	**p34**	**p35**	**p21**
1ERC	1	14	22	19	67	-1	-1	-1
6ERC	1	14	18	19	1013	-1	-1	-1
15ERC	1	14	18	19	67	-1	15	-1
17ERC	1	14	18	19	810	33	310	55
26ERC	1	11	18	19	69	77	1214	44
28ERC	1	-1	-1	-1	-1	-1	68	55
88BOZ	2	14	-1	-1	67	-1	-1	-1
90BOZ	2	14	25	111	66	-1	714	-1
92BOZ	2	14	210	110	66	33	610	48
93BOZ	2	14	22	110	66	311	210	55
95BOZ	2	14	211	19	66	310	810	55
99BOZ	2	-1	-1	-1	-1	311	619	45
119TRL	3	14	210	19	611	-1	-1	-1
124TRL	3	11	18	19	66	-1	1019	-1
126TRL	3	14	22	19	69	-1	-1	-1
128TRL	3	14	210	89	67	33	38	55
133TRL	3	14	210	19	611	33	58	44
135TRL	3	14	22	19	611	36	514	12
136TRL	3	14	210	19	67	311	310	-1

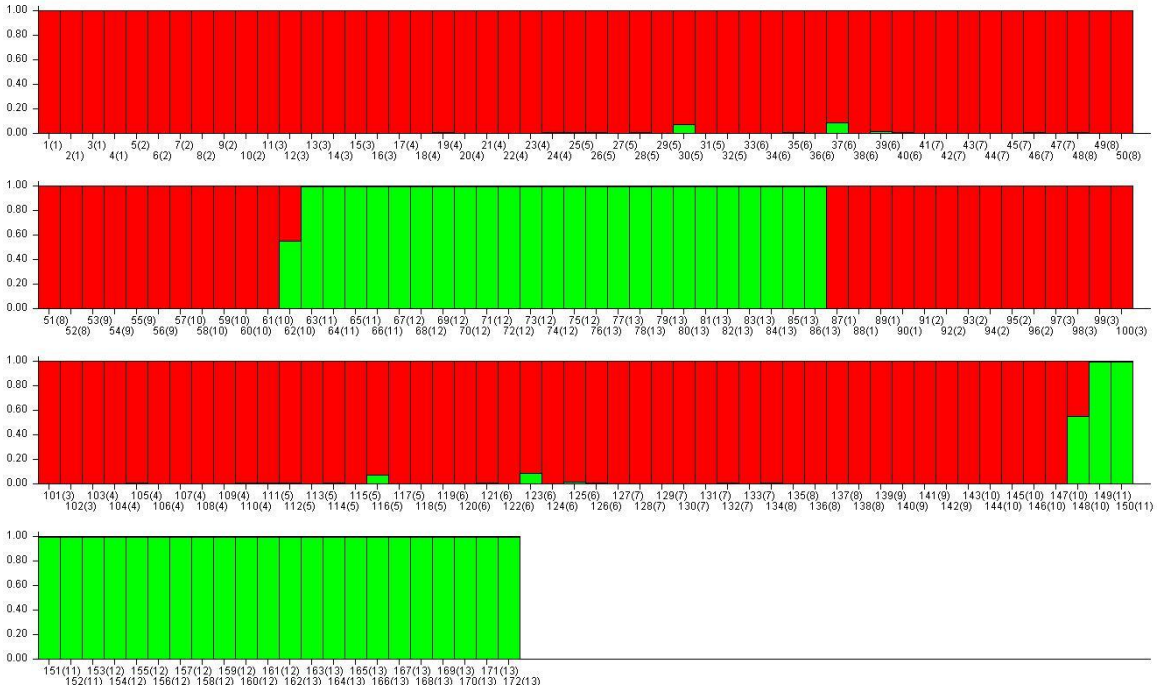
APPENDIX C

An Example File for NTSYS-Pc Programme

	1	**1**	**1**	**1**	**2**	**2**	**2**	**2**	**3**	**3**	**3**	**3**	**4**	**4**	**5**	**5**	**5**	**5**
5	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	1	1	1	0	0	0	0	0	1	1	1	1	1	1	1	1	1	0
39	0	1	1	1	0	0	0	0	1	1	1	1	1	0	0	1	1	999
39	1	0	0	0	0	1	1	1	0	0	0	0	0	0	1	0	0	999
39	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	999
39	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	999
39	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	999
39	0	1	1	1	0	0	0	0	1	1	1	1	1	1	0	1	1	999
9	1	1	1	999	0	1	1	1	1	1	0	1	1	999	1	1	1	999
9	0	0	0	999	0	0	0	0	0	0	0	0	0	999	0	0	0	999
9	0	0	0	999	0	0	0	0	0	0	0	0	0	999	0	0	0	999
9	0	0	0	999	0	0	0	0	0	0	1	0	0	999	0	0	0	999

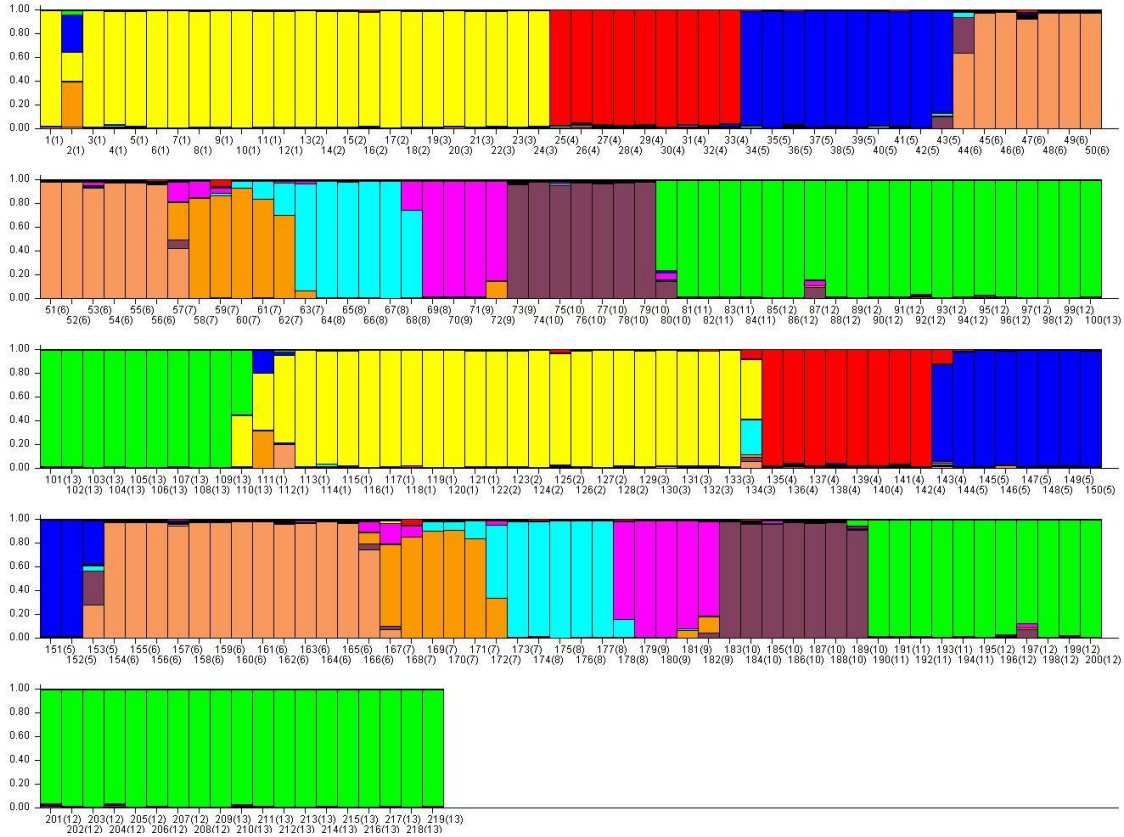
APPENDIX D

The plot of genotypes in multiple lines in their original order (*K*=2)



APPENDIX E

The plot of genotypes in multiple lines in their original order (*K*=9)



APPENDIX F

Allele frequency divergence among populations (Net nucleotide distance),computed using point estimates of P by STRUCTURE software (*K*=9)

1	-	0.2387	0.0852	0.1528	0.0804	0.0742	0.0831	0.1187	0.1330
2	0.2387	-	0.1609	0.2629	0.2051	0.2101	0.2224	0.2190	0.1901
3	0.0852	0.1609	-	0.1185	0.0641	0.0760	0.0731	0.0973	0.0782
4	0.1528	0.2629	0.1185	-	0.1063	0.1250	0.1016	0.1421	0.0920
5	0.0804	0.2051	0.0641	0.1063	-	0.0453	0.0683	0.0762	0.0824
6	0.0742	0.2101	0.0760	0.1250	0.0453	-	0.0765	0.0797	0.0998
7	0.0831	0.2224	0.0731	0.1016	0.0683	0.0765	-	0.1059	0.0959
8	0.1187	0.2190	0.0973	0.1421	0.0762	0.0797	0.1059	-	0.1125
9	0.1330	0.1901	0.0782	0.0920	0.0824	0.0998	0.0959	0.1125	-

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ACADEMIC and PROFESSIONAL EXPERIENCE

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2009-2010	Turkish Atomic Energy Authority Sarayköy Nuclear Research and Training Center	Biologist
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PUBLICATIONS and PREPRINTS

Tümbilen, Y., Frary, A., Mutlu, S., Doğanlar, S., Genetic diversity in Turkish eggplant (*Solanum melongena*) varieties as determined by morphological and molecular analyses, International Research Journal of Biotechnology, 2(1): 16-25, 2011.

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ORAL PRESENTATIONS and POSTERS

Acar, P., **Tümbilen Özer, Y.** And Kaya, Z., Phylogenetic relationships of some Turkish Salix sp. inferred from MatK Sequence Data. In: 3rd International Symposium on Biology of Rare and Endemic Plant Species, Apr 2014, Antalya, Türkiye (Oral Presentation)

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AWARDS and HONORS

TUBITAK (The Scientific and Technical Research Council of Turkey) 2211 – National Scholarship Programme for PhD Students

TUBITAK (The Scientific and Technical Research Council of Turkey) 2210 – National Scholarship Programme for MSc Students

HOBBIES

Folk dances, Movies, Tennis, Outdoor Activities