COMPARATIVE BIOSYSTEMATIC STUDY ON THE *ILEX* Loudon. SECTION OF *QUERCUS* L. (FAGACEAE) SPECIES IN TURKEY

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SEPTEMBER 2011

COMPARATIVE BIOSYSTEMATIC STUDY ON THE *ILEX* Loudon. SECTION OF *QUERCUS* L. (FAGACEAE) SPECIES IN TURKEY

by

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THESIS SUBMITTED TO

THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

OF

THE ABANT İZZET BAYSAL UNIVERSITY

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN

THE DEPARTMENT OF BIOLOGY

SEPTEMBER 2011

Approval of the Graduate School of Natural and Applied Sciences

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ABSTRACT

COMPARATIVE BIOSYSTEMATIC STUDY ON THE *ILEX* Loudon. SECTION OF *QUERCUS* L. (FAGACEAE) SPECIES IN TURKEY

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Ph.D. Thesis, Department of Biology

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September 2011, 108 pages

Evergreen *Quercus* taxa are placed in *Ilex* section. This section contains *Q. ilex*, *Q. coccifera* and *Q. aucheri* which is distributed only in Turkey, and in some East Aegean islands of Greece. However, *Q. coccifera* and *Q. aucheri* usually confused with each other. For this reason, species boundary of *Q. aucheri* and its biosystematic features are not known well. On the other hand, *Q. coccifera* and *Q. calliprinos* are accepted as different species by some researchers or sometimes they can be referred as the same species but two subspecies. Thus, the real phenetics and phylogenetics relations among these species have not still been fully explained.

Due to having hybrids among the taxa which belong to *Ilex* section and complex vegetative variations cause problems and make difficult to determine the borders of taxa. In order to solve this problem, variations within and between populations of taxa were pointed out by using some statistical analyses. According to the results of

statistical analyses, it was attempted to draw the most possible borders of taxa. Furthermore, those vegetative characters which show high correlation with each other were selected and involved in morphometric analyses. As a result, a phenetic classification, which based on vegetative characters, was carried out. Likewise, a molecular but phenetic classification was produced based on the DNA bands obtained from RAPD analyses too. Finally; comparison within and among species was made with cytogenetic study. Congruence between morphology, molecular, and cytogenetic results was compared to make a better phenetic classification.

Key words: *Quercus*, Section *Ilex*, Biosystematics, Cytogenetic, RAPD.

ÖZET

TÜRKİYE *QUERCUS* L. (FAGACEAE) TÜRLERİNİN *ILEX* SEKSİYONU ÜZERİNE KARŞILAŞTIRMALI BİYOSİSTEMATİK ÇALIŞMA

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Eylül 2011, 108 sayfa

Herdem yeşil meşeler *Ilex* seksiyonu içerisinde değerlendirilir. Bu seksiyon *Q.ilex*, *Q. coccifera* ve sadece Türkiye ile Yunanistan'ın bazı Ege adalarında bulunan *Q. aucheri* ile temsil edilir. Bununla birlikte *Q. coccifera* ve *Q. aucheri* genellikle birbirleriyle karıştırılır. Bu nedenle *Q. aucheri*'nin tür sınırı ve biyosistematik özellikleri çok iyi bilinmemektedir. Diğer taraftan *Q. coccifera* ve *Q. calliprinos* bazı araştırmacılara göre farklı türler olarak kabul edilirken bazen de aynı türün iki alt türü olarakta değerlendirildiği olur. Dolayısıyla, bu türler arasındaki gerçek fenetik ve filogenetik ilişkiler hala açıklanabilmiş değildir.

Seksiyonu oluşturan yurdumuzdaki taksonların birbirleri arasında yaptıkları melezleşmeler ve karmaşık vejetatif varyasyonlar takson sınırlarının belirlenmesinde sorun oluştururlar. Bu taksonomik problemlerin çözümü populasyonlar içi ve populasyonlar arası varyasyonların istatistiksel analizleri yapılarak ortaya konuldu. Elde

edilen analiz sonuçlarına göre taksonların olası sınırlarının tespit edilmesine çalışıldı. Ayrıca, aralarında yüksek korelasyon gösteren vejetatif karakterler seçilerek morfometrik analizlere dahil edildi. Sonuçta vejetatif karakterlere dayanan fenetik bir sınıflama ortaya konuldu. Aynı şekilde, RAPD analizlerinden elde edilen DNA bantlarına dayanan moleküler fakat fenetik bir sınıflama da yapıldı. Son olarak sitogenetik çalışma ile tür içi ve türler arası karşılaştırma yapıldı. Daha iyi bir fenetik sınıflama yapabilmek için, bu üç çalışmanın arasındaki uyumluluklar karşılaştırıldı.

Anahtar Sözcükler: *Quercus*, *Ilex* seksiyonu, Biyosistematik, Sitogenetik, RAPD.

ACKNOWLEDGEMENTS

I would like first to thank my supervisor Assist. Prof. Dr. Emel Uslu and my co-supervisor Prof. Dr. M. Tekin Babaç whose gave me support through all stages of my thesis. This study can not be carried out without their pioneering.

I would like to thank my committee members, Prof. Dr. Ekrem Gürel, Prof Dr. Ahmet Şahin and Prof Dr. Yasin Altan.

I also would like to thank to Instructor Cem Koçak, Res. Ass. Necmiye Şahin and Res. Ass. M. Tolgahan Hakan for technical support and also for their friendship.

Finally, I would like to thank my family for their constant support and encouragement.

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ABBREVIATIONS

A_1	Intrachromosomal asymmetry
A_2	Interchromosomal asymmetry
Bp	Base-pair
CA	Cluster Analysis
cpDNA	Chloroplast DNA
dATP	Deoxyadenosine triphosphate
dCTP	Deoxycytidine triphosphate
dGTP	Deoxyguanosine triphosphate
DNA	Deoxyribonucleicacid
dTTP	Deoxythymidine triphosphate
I^c	Centromeric index
L/S	Largest/Shortest
mtDNA	Mitochondrial DNA
PC	Plant Binding Buffer
PCA	Principal Component Analysis
PCR	Polymerase Chain Reaction
PE	Plant Elution Buffer
PL_1-PL_3	Plant Lysis Buffer
PW_1-PW_2	Plant Wash Buffer
RAPD	Random Amplified Polymorphic DNA
Taq	Thermus aquaticus
UPGMA	Unweighted Pair-Group Method using Arithmetic Average

CHAPTER I

1. INTRODUCTION

1.1. *QUERCUS*

Oaks in North Hemisphere are the most outstanding species of deciduous forests in temperate zone. Govaerts and Frodin (1998) state that the genus *Quercus* is represented by 531 species in the world and 250 of these species in America, 125 of these in Asia and remaining species in Europe, North Africa and Macaronesia have habitats. Especially; it is considered which the region containing South East Asia and Pacific islands is the center of morphological variation, at the same time; this region is the origin center of Fagaceae (Kaul, 1985).

It is propounded that the genera of Fagaceae family emerged in cretaceous and then, the genera belonging to Fagaceae family fastly evolved in temperate zone (Axelrod, 1983). *Quercus* species show the most variation in Sierra Madre located in the West Mexico and in East and South East Asia (Govaerts and Frodin, 1998).

Oaks are the woody, long-lived and wind pollination species. For this reason, oaks can spread too wide geographic regions and as a result of this, they show high variations (Kremer and Petit, 1993; Hokanson et al., 1993; Bacilieri et al., 1996). Because breeding barrier between *Quercus* species are very week, oaks living in mixed populations belonging to same or not same sections show the hybridization behaviors (Bacilieri et al., 1996; Manos et al., 1999; Samuel, 1999). In other word, oaks living in

the same region are hybridized with each other and consequently, hybrid species spring up. Then these hybrid species make back crossing with parent species (Burger, 1975). In addition to; self fertilization is not observed in oaks and this situation is provided with self incompatibility system in oak species. Consequently, the presence of self incompatibility system raise the hybridization and taxonomic problems in oaks (Bacilieri et al., 1996) but this event can show the difference from one species to other (Müller-Starck et al., 1996). Only one oak tree can produce millions pollens and this is the most important features of the oaks. Pollens belonging to oaks can spread to thousands kilometers (Petit et al., 1997). Due to, these features about oaks, most of species in Turkey and all distributed countries have taxonomic problems. To solve taxonomic problems and to determine the limits of taxa is necessity (Borazan and Babaç, 2003). In addition to; other an important reason of the variation between oak species can be differences in geographical region (Petit et al., 1997). The presence of morphological variations in the same species living different geographical regions having distinct ecological factors is usual. To introduce the reasons of the variations is very important. Variations can be caused ecological or genetical factors (Davis and Heywood, 1963).

The taxonomy of genus *Quercus* is based on morphological traits and these traits are still crucial to differentiating species within the genus *Quercus* (Dupouey and Badeau, 1993; Bruschi et al., 2000). Leaf characters are very important in the classification of oaks (Stace, 1989) and in the determination of the limits of species (Jensen et al., 1984). Leaf characters are using for classifying of most oaks. They are also one of the most important indicators in identifying hybrid samples. According to the Stace (1989), one of the most important characteristic features of hybrids is

asymmetric and formless leaves. When the characters of flower and reproduction organs are deficient, vegetative characters are used involuntary (Stace, 1989). As a result of hybridization, it is known that quantitative leaf characters are more susceptible to variation in the comparison to quantitative fruit and bud characters (Jensen, 1988 and 1989). It is well known that most tree species produce leaf size in a wide range depending upon seasonal and positional effects. Ligh density influences leaf morphology. For example, the leaves on outer branches have broader, deeper sinuses and a narrower lamina than inner leaves. This shade effect is common in oaks. Therefore it is recommended that leaf collection in taxonomic and stystematic studies should be on the same date and after leaf growth has stopped and from the same height and location to avoid taxonomical errors (Blue and Jensen, 1988).

Untill now; generally morphological and anatomic characters have been used in the phenetics and phylogenetic classification of oaks and at the present day, these characters have been still used (Jensen, 1988). Generative characters are deficient in the classification. Furthermore vegetative characters display too much variations. There are also much hybrid samples in the oaks. As a result of these problems, to find new characters for classification is necessity. In other word; recently, molecular characters are used for the phenetics and phylogenetic classifications. Especially isozyme and molecular characters are preferring often for the phenetics and phylogenetic classification (Hokanson et al., 1993; Samuel et al., 1995; Müller-Starck et al., 1996).

Molecular techniques are usually preferred to explain genetics status of taxa, also in the phylogeny and biogeographical distributions. Especially PCR (Polymerase Chain Reaction) method is used to solve taxonomic problems caused from morphological classification (Hillis et al., 1996; Soltis et al., 1998; Mullis and Falcona,

1987; Wolfe and Liston, 1998). Chloroplast DNA (cpDNA) markers are used widely in oaks for the determination of geographical distribution among populations (Petit et al., 2002a; Kremer et al., 1991). CpDNA is the preferred cytoplasmic marker in plants because genetic material of chloroplast is transfered as maternal like many angiosperms. CpDNA is protected from affect of recombination (Dumolin et al., 1995; Soltis et al., 1992). CpDNA variation in oaks are used for the determination of burrow fields after glacier period and in the determination of geographical distributions for the phylogenetic research (Petit et al., 2002a; Petit et al., 2002b; Fineschi et al., 2002; Bordacs et al., 2002).

These molecular techniques are using in the classification of many oak taxa in the world (Moreau et al., 1994; Dumolin et al., 1995; Bacilieri et al., 1996; Petit et al., 2002a; Petit et al., 2002b).

Characters resistant to variations have the biggest role in the classification and determination of taxa. For this reason; studies in the molecular level in oaks are focused on cpDNA, mtDNA (Mitochondrial DNA) and RAPD (Random Amplified Polymorphic DNA) (Romero-Severson et al., 2003; Jimenez et al., 2004; Buiteveld and Koelewijn, 2006; Lumaret et al., 2002; Petit at al., 2002a; Petit at al., 2002b; Csaikl et al., 2002; Dumolin-Lapegue et al., 1998; Lopez-de-Heredia et al., 2005; Lumaret et al., 2005). For example, genetic variations and differences among populations in *Quercus affinis* Schweid and *Quercus laurina* Humb&Bonpl. species belonging to red oaks were studied using RAPD markers by Gonzalez-Rodriguez et al. (2005). In the same way; genetic variation of *Quercus robur* in Russia were studied using RAPD markers by Yakovlev and Kleinschmidt (2002).

1.1.1. OAKS IN TURKEY

Turkey is one of the most important region of the world according to oak species number and variation. At present; oaks are represented by 18 species (Davis, 1982; Yaltırık, 1984; Kasapligil, 1992) and these are included in three sections in the Flora of Turkey (Davis, 1982) as white oaks (*Quercus* L.), red oaks (*Cerris* Loudon.) and evergreen oaks (*Ilex* Loudon.) (Table 1). After the study of "Revision of Oaks in Turkey", number of the taxa is decreased from 35 to 23 by Hedge and Yaltırık, 1982. In the last classification published in the Flora of Turkey, still there are various nomenclatural and typification problems (Hedge and Yaltırık, 1982).

It is well known that reproductive barriers between oak species are very week (Bacilieri et al., 1996; Manos et al., 1999; Samuel, 1999). Furthermore; hybridization may occur extensively between species in the same major group or section. Oak species have intra specific morphological variation due to hybridization (Manos et al., 1999). This situations cause many taxonomic problems in our country like other countries.

Evidences provided from studies show that in glacial period, many animals and plants retained their lives in the burrow of Turkey (Hewitt, 1996 and 1999). Consequently; it is considered that Turkey is the center of genetic origins for many species of Europe. Therefore it is important to know oak species, their origins, biogeographic distributions, etc.

Table 1- Taxonomic hierarchy of genus *Quercus*

Kingdom Plantae (Plants)
Subkingdom Tracheobionta (Vascular plants)
Division Magnoliophyta (Angiosperms)
Class Magnoliopsida (Dicotyledons)
Subclass Hamamelidae
Order Fagales
Family Fagaceae
Genus *Quercus* L. (Oaks)

Section *Quercus* (Endl.) (White oaks)
Q. pontica C. Koch
Q. robur L.
    Q. robur subsp. *robur*
    Q. robur subsp. *pedunculiflora* (C. Koch) Menitsky
Q. hartwissiana Steven
Q. macranthera subsp. *syspirensis* (C. Koch) Menitsky
Q. frainetto Ten.
Q. petraea (Mattuschka) Liebl.
    Q. petraea subsp. *petraea*
    Q. petraea subsp. *iberica* (Steven ex Bieb.) Krassiln.
    Q. petraea subsp. *pinnatiloba* (C. Koch) Menitsky
Q. vulcanica (Boiss & Heldr. Ex) Kotschy
Q. infectoria Oliver
    Q. infectoria subsp. *infectoria*
    Q. infectoria subsp. *boissieri* (Reuter) O. Schwarz
Q. pubescens Willd.
Q. virgiliana Ten.

Section *Cerris* Loudon (Red Oaks)
Q. cerris L.
    Q. cerris var. *cerris*
    Q. cerris var. *austriaca* (Willd.) Loudon
Q. ithaburensis subsp. *macrolepsis* (Kotschy) Hedge and Yalt.
Q. brantii Lindley
Q. libani Oliver
Q. trojana P.B. Webb

Section *Ilex* Loudon (Evergreen Oaks)
Q. ilex L.
Q. coccifera L.
Q. aucheri Jaub. & Spach

1.1.2. MORPHOLOGY OF *QUERCUS*

The description of genus *Quercus* is as following in the Flora of Turkey (Davis, 1982) by Hedge and Yaltırık;

> "Deciduous or evergreen trees, rarely shrubs; buds spirally arranged, with imbricate scales, clustered at shoot apices. Leaves subsessile or petiolate, penninerved, serrate, dentate, pinnatifid or lobed, lobes rounded without bristles at their tips or sharply pointed with aristate tips, rarely entire. Staminate flowers in long slender pendulous catkins; calyx (4-)6(-7)- partitate; stamens (4-)6(-12). Pistillate flowers solitary or 2 to several on a very short to fairly elongate peduncle; ovary 3(-4)- celled. Fruit a nut (acorn), subglobose to oblong or cylindrical, surrounded at base or sometimes nearly enclosed by cup-shaped cupule covered outside with numerous imbricate scales (in Turkish specimes); pericarp thin or thick, endocarp glabrous or pubescent; acorn maturing in one season or two years, sweet or bitter to taste."

1.1.3. EVERGREEN OAKS

Ilex section is represented by three species in Turkey known as *Quercus coccifera*, *Quercus aucheri* and *Quercus ilex*. Evergreen oaks section is also having problematic species. Such as; *Quercus aucheri* only exist in the South West Anatolia region of Turkey and in the some Greek islands like Rodos. Therefore; the determination of genetic features of *Q. aucheri* is very important for the European oaks. Due to limitation of this species border, it is called as forgotten oaks (Yaltırık, 1984). Because of *Q. aucheri* exist in restricted area in the world, there is not enough morphological, molecular and cytogenetics study on this species. There is a study only

on the polyphenolic compound of the leaves of *Q. aucheri* and on it's antimicrobial activity (Sakar et al., 2005).

Quercus coccifera accepted as other member of *Ilex* section, and is confused with *Quercus calliprinos* Webb. (Toumi and Lumaret, 2001). Mostly; these taxa show the differences in geographical distribution. While *Q. coccifera* exist in West Mediterranean region, *Q. calliprinos* exist in East Mediterranean region (Toumi and Lumaret, 2001). But Toumi (1995) states in his Ph.D. study that these two species can be evaluated within *Q. coccifera* as *Q. coccifera* subsp. *coccifera* and *Q. coccifera* subsp. *calliprinos*. According to some other reserchers, *Q. coccifera* and *Q. calliprinos* are the two different species, this subject related to *Q. coccifera* is still controversial (Salvatore and Paola, 1976).

The last member of *Ilex* section, *Q. ilex* has also some taxonomic problems like other species of evergreen oaks. *Q. ilex* has two morphological types. These are rotundifolia type containing small and round leaves and ilex type containing big pointed leaves. Rotundifolia morphotype exist in North Africa and interior region of Spain (Tutin et al., 1964). Ilex morphotype exist along the Atlantic coast of France. These two morphotypes are two different species (Tutin et al., 1964) or two subspecies (Saenz De Rivas, 1967) or only two varieties (Maire, 1961). Additionally; it is reported the presence of intermediate forms for these two morphotypes in the Mediterranean region of France and in the North and East coasts of Spain. Allosyme studies support the presence of one species consist of two subspecies (Michaud et al., 1995).

The effect of human on the evergreen oaks is too little except *Q. suber*. Generally; oaks are used as wood coal, in Spain *Q. ilex* is accepted as fruit tree. Acorns

of *Q. ilex* are used for feedings of pigs. In evergreen oaks, the presence of hybrid samples in mixed populations is reported too (Camus, 1938).

1.2. MOLECULAR TOOLS FOR OAKS

1.2.1. RAPD-PCR METHOD

RAPD (Randomly Amplified Polymorphic DNA) is a PCR based techniques used to show polymorphism among species.

In this method unknown segments of DNA are amplified, i.e. target sequences to be amplified is unknown. Single short oligonucleotide primers containing often 10 bases are used in RAPD-PCR method. These RAPD primers containing short 10 bases generate random fragments from template DNA. These fragments are compared for individuals or for species. This method requires previous testing of many primers (Lee et al., 1996). It allows us to detect sequence changes within PCR priming sites. RAPDs show polymorphism and thus can be used as genetic markers. It can also be used at the population level. RAPDs are dominant markers and allow us to see how many genotypes present in a population. This means the presence of a RAPD band does not allow distinction between heterozygous and homozygous states. However, in some cases, RAPD bands of different lenghts can be assigned to the same locus. In such a case, these RAPD bands are codominant. Especially this method is very helpful for systematics purposes (Bin et al., 1993). RAPD-PCR method is used to show polymorphism and phylogenetic relation (Landry, 1993; Klein-Lankhorst et al., 1991; Caettano et al., 1991).

1.2.2. THE NATURE OF RAPD POLYMORPHISM

Firstly, primer binds to many locations on the template DNA. If two of these locations are close to each other and oriented in opposite direction, amplification is established and this region amplified is called as a RAPD locus. On the contrary, if connected locations of primers are away to each other and oriented in the same direction, amplification does not take place. In other word, the primers anneal within a reasonable distance of one another (150–3000 bp). The number of amplification products is related to the orientation of the genome sequences which are complementary to the primer is presented in Figure 1.

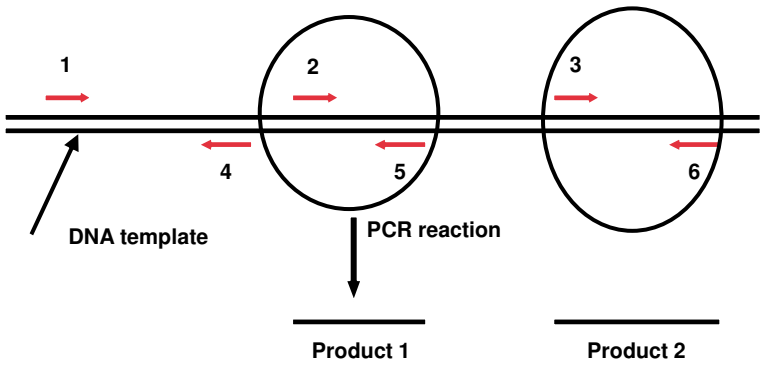

Figure 1- The nature of RAPD polymorphism

Nucleotide substitution within target sites may affect the annealing process. If primers are just the right distance apart, so fragment is amplified and these distance is between 150-3000 bases. If nucleotide substitution is in large-scale, amplification does not take place (Figure 2-a) or product bigger than normal is provided (Figure 2-b).

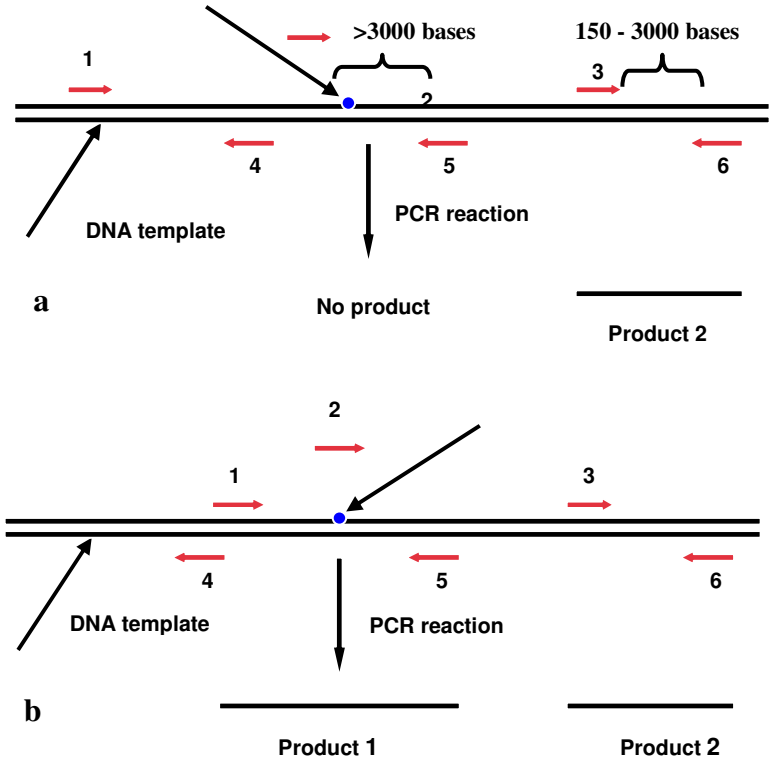

Figure 2- a-b Nucleotide substitution in RAPD polymorphism

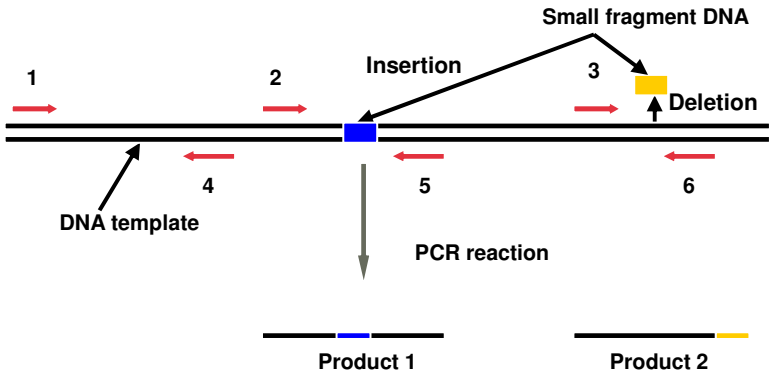

Figure 3- Insertion and deletion in RAPD polymorphism

If insertion or deletion of a small fragment of DNA is occurred, the amplified fragments sizes are changed. The size of product 1 increases because of insertion, while the size of product 2 reduces because of deletion (Figure 3).

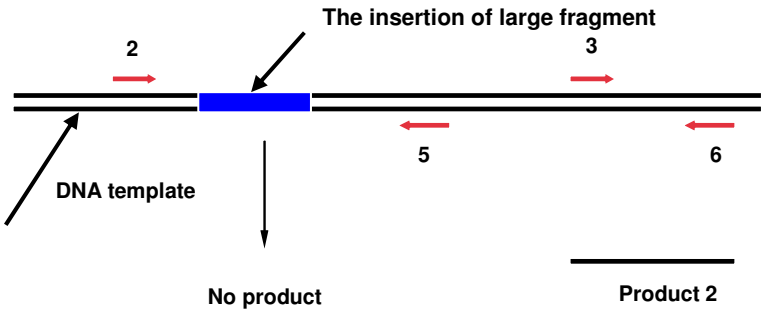

Figure 4- Insertion of a large piece of DNA in RAPD polymorphism

Insertion of a large piece of DNA between the primer-binding sites may exceed the capacity of PCR and as a result of this fragment is not produced (Figure 4).

1.2.3. ANALYSES OF THE RAPD DATA

RAPD produces a large number of DNA bands of various sizes from each of the different samples which are prepared. These bands migrate according to size during electrophoresis. Firstly, the sizes of DNA fragments provided RAPD-PCR for each primer separately, are established in each population. Then the tables are constructed containing number and size of the DNA fragments which produced for each populations. According to these bands monomorphic and polymorphic bands are determined for all populations (Figure 5).

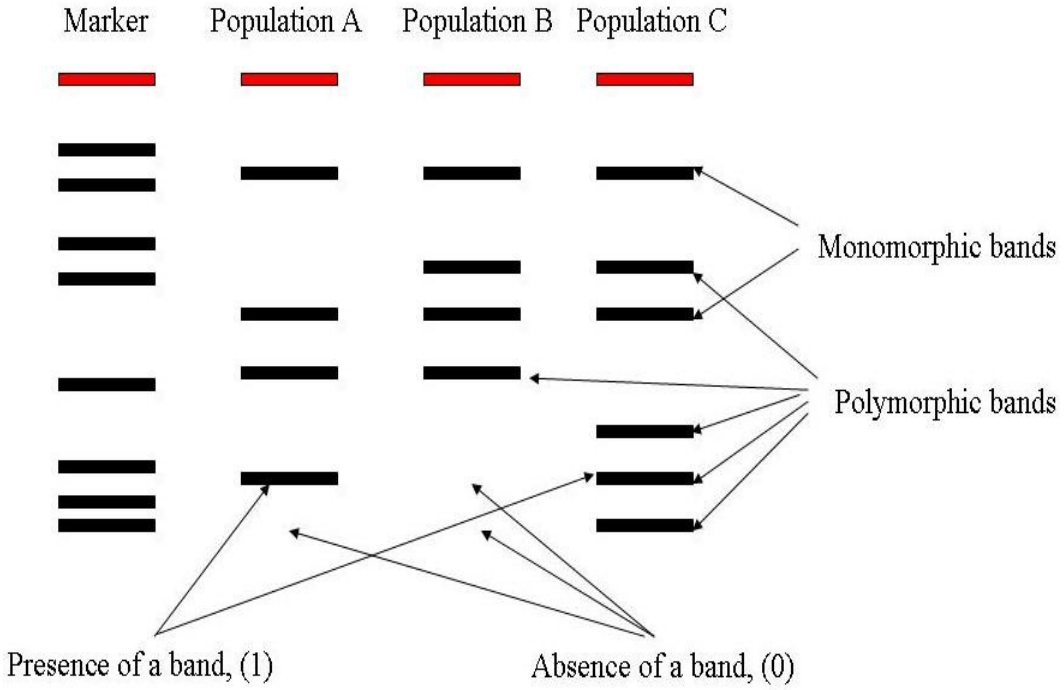

Figure 5- Determination of monomorphic and polymorphic bands

RAPD bands are scored for presence 1 and absence 0. Only clear, consistent and polymorphic bands are usually used to create a binary matrix. In other words, the tables presenting the number and size of the DNA fragments are changed to the codes (Figure 5).

1.3. CYTOGENETIC STUDY

Material collection is a first step of cytogenetic studies. It is crucial to find tissues containing much cell division. For this reason, generally root tips with 1-1,5 mm are preferred for karyotype studies in plants. If root tips grow more, weak useless roots develop and cell divisions in these roots can not observed. Therefore, it is crucial to take root tips in the right time.

Root tips with 1–1,5 mm are put into tubes and pretreated with first treatment solution. Different solution for first treatment can be used but α-monobromonaphthalene

is often preferred (Şahin and Elçi, 1986; Çobanoğlu and Altan, 1989; Engin et al., 1994; Yılmaz et al., 2008; Yılmaz et al., 2009). This solution leads to shorten and thickening of chromosomes. At the same time, spindle strands are terminated by the first treatment solution. As a first treatment, root tips are pretreated in α-monobromonaphthalene for 16–17 hours at 4 ^{o}C. This is standart approaches for this solution. Other solution used for first treatment is paradichlorobenzen. Generally, exposure time of paradichlorobenzen for root tips is 4 hours (Klamit and Schifino-Wittmann, 2000; Şahin, 1993). Alternative first tretament solution is colchicine. It can be used in different concentrations according to the researchers (Demerico et al., 1995; Meriç and Dane, 1999; Loidl, 1985; Yüzbaşıoğlu et al., 1997). The last solution used as first treatment is 8-hydroxyquinoline. Exposure time of 8-hydroxyquinoline for root tips is 2.5 hours (Naranjo et al., 1998).

After the first treatment, root tips is treated with fixative solution. For this aim, alcohol-acetic acid mixture is used but according to the researcher alcohol-acetic acid mixture can be used in different rates (Perisse et al., 2000; Kar and Sen, 1991).

The next step is hydrolize with HCl (hydrochloric acid). This step can be used in different concentrations and temperature (Yüzbaşıoğlu et al., 1997; Gu et al., 1984; Greilhuber, 1998; Demerico et al., 2000).

The final step is staining and slide preparation. Different staining solutions can be used for chromosomes in cytogenetic studies. Aceto-orcein and feulgen are the two staining solutions preferred by many researchers (Martin et al., 2006; Martin et al., 2008; Badr and Sharawy, 2007; Sevimay et al., 2005).

CHAPTER II

2. AIM OF THE STUDY

Oaks are well known with their problematic taxonomies. The genus *Quercus* is characterized by high poymorphism and complex patterns of variation (Burger, 1975; Manos et al., 1999). Hybridization and introgression are often present, even between morphologically and ecologically clearly differentiated species. This makes the taxonomy of the genus very complex and often controversial (Franjic et al., 2006).

Additionaly; taxa belonging to *Ilex* section are not known very well. However; these taxa have taxonomic problems too. There is not enough researches on *Q. aucheri* and other species of *Ilex* section. In addition to; distinction between *Q. coccifera* and *Q. calliprinos* are not very clear. These two taxon's names are used synonymously in some Floras. They may be the same or different taxa. Another problem is that some times *Q. ilex* is included in *Cerris* section (Manos et al., 1999).

In order to solve such problems, following steps are being aimed in this study:

1. to determine the variations within and among species,
2. to determine the genetic distances among species,
3. to compare the results of morphological, molecular and cytogenetic studies with one another,

4. to evaluate the results of morphological, molecular and cytogenetic analysis for phenetic classifications together,

5. to determine taxonomic status of the taxa.

CHAPTER III

3. MATERIALS AND METHODS

3.1. MORPHOLOGICAL STUDIES

Study materials are composed of three species (*Q. coccifera*, *Q. ilex* and *Q. aucheri*) belonging to *Ilex* section of Turkey oaks. These materials were collected in 2008- 2009 years and conserved in the herbarium of Abant Izzet Baysal University.

About 17 populations were designated to reveal variations within *Q. coccifera* (Table 2). In order to represent both coastal region and interior region of *Q. ilex*, about 5 populations were designated (Table 3). *Q. aucheri* is not common like *Q. coccifera* in Turkey. *Q. aucheri* only exists in South West Anatolia region of Turkey and for this reason; it is represented by 5 populations in this study (Table 4). On the contrary of *Q. aucheri*, *Q. ilex* has a wider distribution area.

Morphological studies are compased of four levels. These are;

1. Collection of materials

2. Determination of characters

3. Measurement of characters

4. Evaluation of characters with numerical techniques.

Table 2- Populations of *Q. coccifera* including population number, location, coordinates and altitude

Q.coccifera				
Pop. No	**Location**	**Coordinates**		**Altitude**
		N	**E**	**(m)**
c_1	**İzmir**-Balıkesir border area, Altınova barrage road	$39^0$12.903	$026^0$49.302	70
c_2	**İzmir**-between Dikili-Çandarlı, 20 km. to Çandarlı	$39^0$01.253	$026^0$55.505	40
c_3	**Manisa**-between Kırkağaç-Akhisar, 1-2 km. after Çandarlı	$39^0$05.800	$027^0$40.257	190
c_4	**Çanakkale**-Ezine-Bozcaada pier	$39^0$47.950	$026^0$12.115	50
c_5	**Gökçeada**-between Gökçeada-Dereköy	$40^0$09.689	$025^0$49.586	60
c_6	**Mersin**-5-10 km. after Seratvul	$36^0$50.997	$033^0$18.402	1400
c_7	**Karaman**-between Mut-Ermenek, 45 km. before Ermenek	$36^0$37.276	$032^0$55.182	1300
c_8	**Antalya**-between Korkuteli-Bucak, 25 km. before Bucak	$37^0$15.582	$030^0$19.362	920
c_9	**Aydın**-Eski Çine, Ovacık village	$37^0$32.889	$028^0$05.310	300
c_{10}	**Aydın**-Söke, between Bağarası-Akçakaya village	$37^0$40.350	$027^0$31.347	40
c_{11}	**Muğla**-between Muğla-Kale, 59 km. before Kale	$37^0$08.142	$028^0$32.157	800
c_{12}	**Denizli**- between Kale-Tavas, 1-2 km. before Tavas	$37^0$33.069	$029^0$03.150	940
c_{13}	**Uşak**-between Sivaslı-Uşak, 12 km. after Sivaslı	$38^0$34.259	$029^0$36.303	825
c_{14}	**Yalova**- between Çınarcık-Armutlu,	$40^0$38.220	$029^0$03.688	170
c_{15}	**Gaziantep**- between Yavuzeli-Araban	$37^0$22.975	$037^0$33.292	740
c_{16}	**Kahramanmaraş**- between k.maraş- göksun	$37^0$43.514	$036^0$40.038	1075
c_{17}	**Hatay**-between Kırıkhan-Hassa	$36^0$36.554	$036^0$23.591	350

Table 3- Populations of *Q. ilex* including population number, location, coordinates and altitude

Q.ilex				
Pop. No	**Location**	**Coordinates**		**Altitude**
		N	**E**	**(m)**
i_1	**Zonguldak-**Alaplı, Sabırlı village	$41^0$08.901	$031^0$23.147	180
i_2	**Zonguldak-**between Alaplı-Düzce	$41^0$08.443	$031^0$20.596	4
i_3	**Düzce-** between Yığılca-Alaplı	$41^0$09.136	$031^0$23.627	60
i_4	**İstanbul-**between Anatolian Fortrees-Kavacık	$41^0$04.220	$029^0$05.085	65
i_5	**Gökçeada-**between Gökçeada-Dereköy	$40^0$09.689	$025^0$49.586	60

Table 4- Populations of *Q. aucheri* including population number, location, coordinates and altitude

Q.aucheri				
Pop. No	**Location**	**Coordinates**		**Altitude**
		N	**E**	**(m)**
a_1	**Antalya-**between Kemer-Kumluca	$36^0$25.429	$030^0$25.447	530
a_2	**Aydın-**Çine, Across from the cemetery Kuruköy	$37^0$33.558	$028^0$04.047	180
a_3	**Aydın-**Priene-Söke	$37^0$44.967	$029^0$16.369	90
a_4	**İzmir-**Selçuk-Zeytinköy	$37^0$59.569	$027^0$17.226	65
a_5	**Muğla-**between Milas-Bodrum, Dörttepe village	$37^0$11.242	$027^0$37.142	8

3.1.1. COLLECTION OF MATERIALS

Leaves and fruits were used as material to show the differences in the morphological study. *Q. coccifera* is represented by 17 populations and other two species (*Q. ilex* and *Q. aucheri*) are represented by 5 populations. In other word; totally 27 populations were determined for these three species (Figure 6).

Each population was represented by 10 trees and each tree was represented by 10 samples. Concisely, totally 270 trees and 2700 leaves and fruits were used for morphological part of the study. Leaf and fruit materials were collected from adult trees at least 100 m apart for the most accurate representation of the population.

In order to eliminate the shade effect (Borazan and Babaç, 2003; Blue and Jensen, 1988) all leaf samples were collected at approximately the same level and location after leaf growth had stopped to avoid seasonal and positional variations.

For fruits, mature acorns with regular shapes were chosen while collecting from trees randomly. During the sample collection, acorns were chosen with cupules, since cupules were also used for character selection. Collected acorns put into paper bags and then allowed to dry in a place with no humid. Each paper bag represents a tree and labeled with its population and tree number.

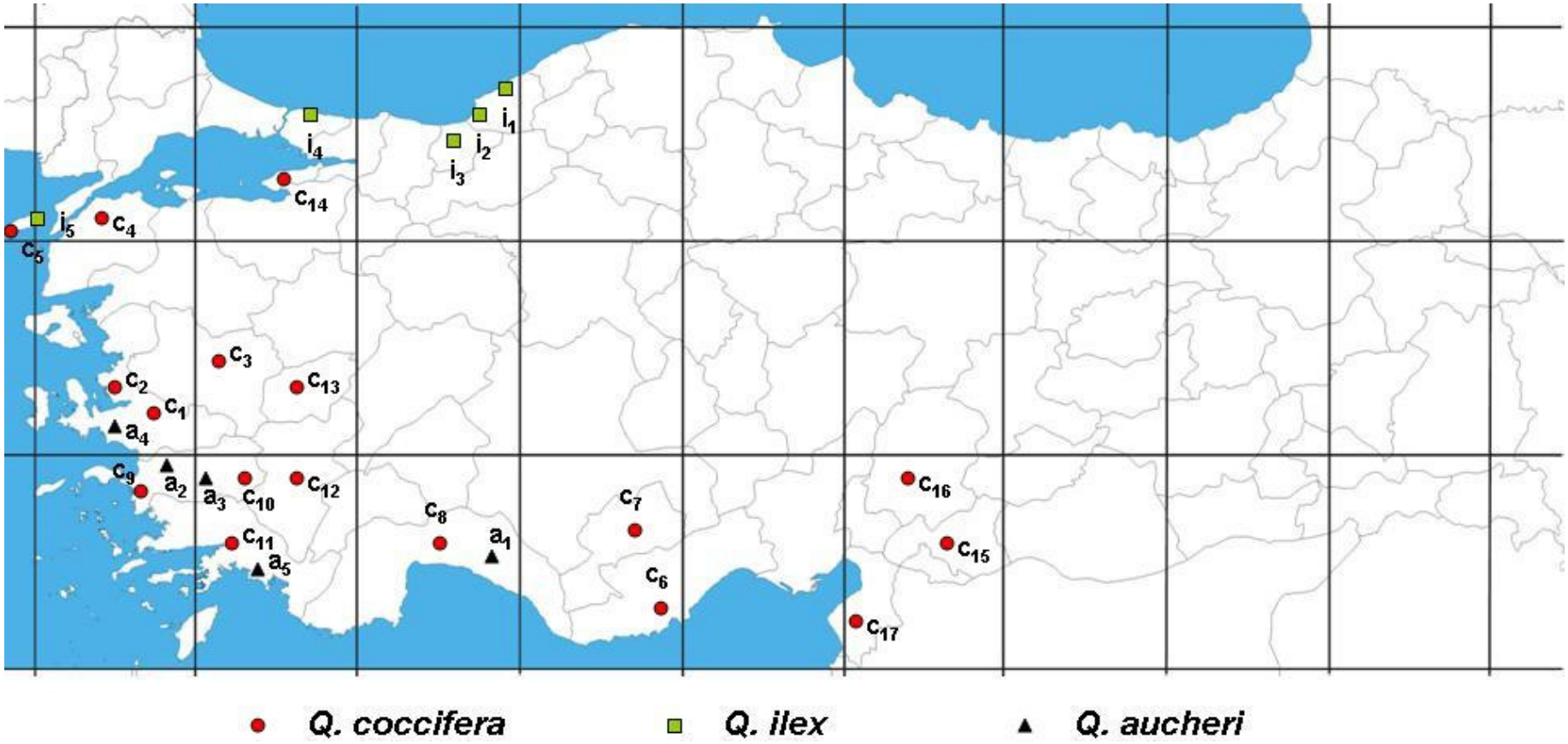

Figure 6- Distribution of studied populations of *Q. coccifera*, *Q. ilex* and *Q. aucheri* in Turkey

3.1.2. DETERMINATION OF CHARACTERS

Totally twenty five characters from leaves and fruits were used in this study. While ten of the selected characters belong to the leaf (Figure 7) (Table 5), remaining fifteen characters belong to the fruits (Figure 8) (Table 6). All of the selected characters are quantitative. Since quantitative characters are more susceptible to change in the comparison to qualitative characters.

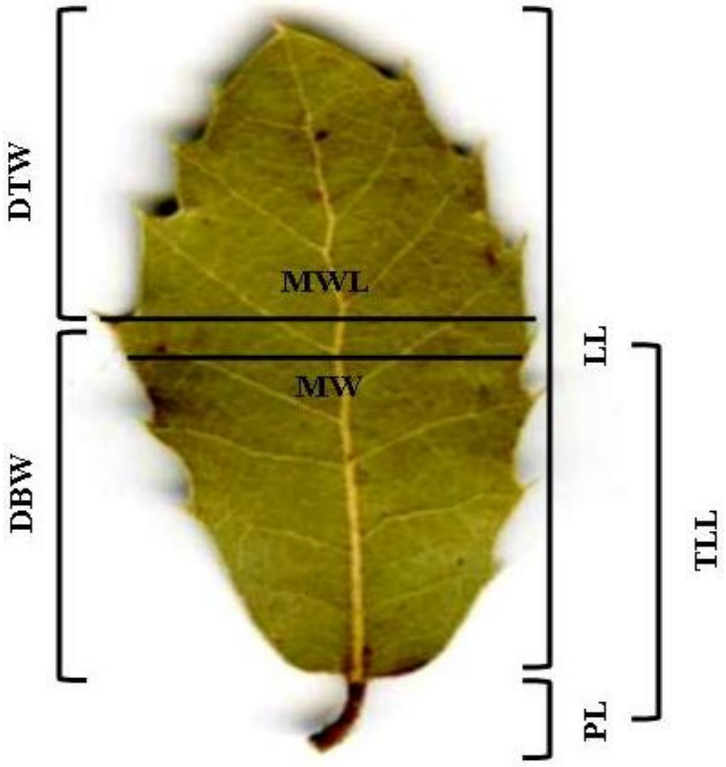

Figure 7- Morphological leaf characters

Figure 7 shows the quantitative seven characters used for leaves. The rest characters used for leaves are index characters and these are shown in Table 5 with other characters. Many of leaf characters were adopted from different sources (Borazan and Babaç, 2003; Bruschi et al., 2000; Kremer et al., 2002; Boratynski et al., 2008;

Gonzalez-Rodriguez et al., 2004; Bruschi et al., 2003; Franjic et al., 2006; Ponton et al., 2004; Gonzalez-Rodriguez and Oyama, 2005).

Table 5- Leaf characters used in the morphological analyses

LL: Lamina length
PL: Petiole length
MWL: Maximal width of lamina
MW: Middle width of lamina
DTW: The distance between the widest point and the leaf tip
DBW: The distance between the widest point and the leaf base
TLL: Total leaf length (LL+LP)
P%: Petiole length (PL) x 100/total leaf length (TLL)
MW%: Middle width of lamina (MW) x 100/total leaf length (TLL)
MWL%: Maximal width of lamina (MWL) x 100/total leaf length (TLL)

Totally 15 characters were used for fruits and these are 3 characters from nut, 5 characters from cupule, 2 characters from stalk, 4 characters from index and the last character from acorn mass.

Fruit characters were selected and determined from different researches. (Nikoliç and Orloviç, 2002; Tilki and Alptekin, 2005; Bakış, 2005). Figure 8 represents the 10 characters provided from nut, cupule and stalk. Others are acorn mass and index characters (Table 6).

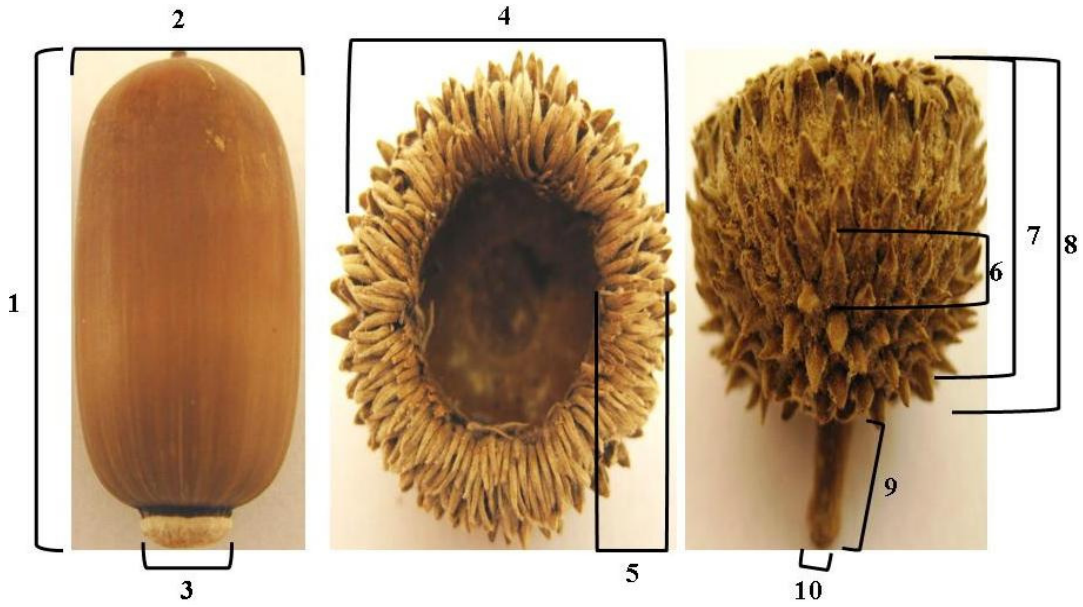

Figure 8- Morphological fruit characters with character number

Table 6- List of fruit characters used in the morphological analyses

Nut characters
1. Nut length
2. Nut diameter
3. Nut scar diameter
Cupule characters
4. Cupule outer diameter
5. Cupule thickness
6. Cupule scale length (maximum)
7. Cupule depth
8. Cupule length
Stalk characters
9. Stalk length
10. Stalk thickness
Index characters
11. Cupule length / Nut length
12. Cupule depth / Cupule length
13. Nut diameter / Nut length
14. Cupule thickness / Cupule outer diameter
15. Acorn mass

3.1.3. MEASUREMENT OF CHARACTERS

This study was made on 27 populations belonging to three species. Each populations were represented by 10 trees and for each tree 10 leaves and fruits were collected from different branches to avoid statistical errors. In other word, each populations were represented by 100 samples for the determination of variations of leaves and fruits. All characters were measured with digital caliper to eliminate the error.

Firstly, arithmetic means of all trees were calculated for each character, then, means of the populations were calculated for each characters, finally; the tables were formed presenting the means of populations for each character in both fruit and leaf samples.

3.1.4. EVALUATION OF CHARACTERS WITH NUMERICAL TECHNIQUES

In fruit, the means value for each character were used to form X-Y scatter diagram and then all of the populations for each characters were compared by using this diagram. The coding was made by comparison of X-Y diagram for each population. However, the raw data were used for leaves instead of coding data. This difference is caused by which in oaks leaf characters are more valuable in the comparison for quantitative fruit and bud characters (Jensen, 1988 and 1989). Beside this, it is known that quantitative leaf characters are more susceptible to variation and good indicator of hybridization.

Analysis of variations in leaf and fruit samples were carried out by using Principal Component Analysis (PCA) and Cluster Analysis (CA) using Statistica version 8.0. Multivariate techniques are used in analysis because these techniques allow

comparing samples taken from different localities or from what are belived to be different taxa. Eventual diagrams showing the variations and similarities within and among populations representing species were formed by using PCA and CA methods.

3.2. MOLECULAR STUDIES

For molecular studies; leaves collected from 27 populations were used as material (Table 2, 3 and 4). Especially, fresh leaves were preferred as material. Collected leaves were put into plastic bags filled silica gel and dried for the DNA isolation. Each population was represented by 6 trees.

After the material collection, molecular study was performed in four levels. These are;

1. DNA isolation
2. RAPD-PCR method
3. DNA gel electrophoresis and visualization
4. Evaluation of populations with numerical techniques.

3.2.1. DNA ISOLATION

Many different methods and technologies are available for DNA isolation. In general, all methods involve disruption and lysis of the starting material followed by the removal of proteins and other contaminants and finally recovery of the DNA. Removal of proteins is typically achieved by digestion with proteinase K, followed by salting-out, organic extraction, or binding of the DNA to a solid-phase support (either anion-exchange or silica technology). DNA is usually recovered by precipitation using ethanol

or isopropanol. The choice of a method depends on many factors: the required quantity and molecular weight of the DNA, the purity required for downstream applications, the time and cost.

For this study DNA isolation was made in two different ways. In the method of Dumolin-Lapegue et al. (1995), fresh leaf materials dried in silica gel was used. In this method, firstly according to modified CTAB cetyltrimethylammonium bromide / dichloromethane protocol, leaf materials dried in silica gel were processed with CTAB buffer (2% CTAB 2 gr; 1.4 M NaCl 28 ml of 5 M NaCl or 8.19 gr; 100 mM Tris-HCl pH 8.0 10 ml of 1 M; 20 mM EDTA 4 ml of 0.5 M; 1% PVP-40 1 gr; 0.2% 2-mercaptoethanol 200 ml). Afterward centrifuge was made with materials like chloroform, lithium-acetate (LiAc) (4M), ethanol, sodium acetate (NaAc) (3M, at pH 5.2), dichlorometane, phenol / chloroform (1:1), RNAse (10 mg/mL). DNA processed with isopropanol and ethanol was solved in TE solution (10 mM Tris; 0.1 mM EDTA).

As a second method, plant DNA isolation kits were used for the DNA isolation.

The modified kit protocol is as follows;

- Leaf material was powdered with liquid nitrogen in morter and then transfered to eppendorf tubes.
- For each sample 400 µl Buffer PL_1 was added and then 10 µl RNase.
- Mixture was incubated in waterbath at 65^{o}C for 20 min.
- The tubes were shaken every 5 min.
- After this step, 75 µl Buffer PL_3 was added to mixture.

- The mixture was incubated for 10 min. on ice.
- The tubes were centrifuged at 13000 rpm for 10 min.
- Only the upper phase was transferred to new tubes containing filter.
- The tubes were centrifuged at 11000 rpm for 4 min.
- Buffer PC (450 µl) was added.
- The tubes were shaken gently for 1 min.
- After that, 600 µl of sample was taken into new tubes containing filter called as Plant II column.
- These tubes were centrifuged at 11000 rpm for 3 min. and discarded the flow-through.
- This step was repeated for two times.
- Buffer PW_1 (400 µl) was added then tubes were centrifuged at 11000 rpm for 3 min. and discarded the flow-through.
- Buffer PW_2 (600 µl) was added.
- The tubes were centrifuged at 11000 rpm for 3 min. and discarded the flow-through.
- Then 200 µl Buffer PW_2 was added for second time. The tubes were centrifuged at 11000 rpm for 4 min. and again discarded flow-through.
- Finally; 50 µl Buffer PE (70^oC) was added and incubated for 10 min. at 70^oC.

- The tubes were centrifuged at 11000 rpm for 2 min. to elute

- Extracted DNAs were kept at 4^{o}C.

DNAs were controlled by running on agarose gel before used in PCR.

3.2.2. RAPD-PCR METHOD

In this study totally 30 primers, studied in oaks previously, were selected to find primers that exhibit polymorphism and give reproducible results. After the initial screening, 10 primers giving the best results among from 30 primers were selected for further analysis. The list of selected primers and their sequences is given in Table 7.

Amplification reactions were carried out in a 25 µl mix containing 50 ng template DNA, 1X PCR Buffer, 2,5 mM $MgCl_2$, 100 µM dATP, dCTP, dGTP and dTTP, 0,36 µM 10- base RAPD primer and 0,75 unit of taq DNA polymerase.

After the primer selection, PCR conditions was determined according to previous studies on oaks (Gonzalez-Rodriguez et al., 2004; Franjic et al., 2006; Kelleher et al., 2005). Adjusted PCR conditiones are given in Table 8.

Table 7- The list and their sequences of primers used in RAPD analyses

Primers	**Sequence (5^{l}-3^{l})**
OPA- 01	CAGGCCCTTC
OPA- 08	GTGACGTAGG
OPA- 09	GGGTAACGCC
OPB- 04	GGACTGGAGT
OPX- 04	CCGCTACCGA
OPC- 03	GGGGGTCTTT
OPC- 09	CTCACCGTCC
OPS- 09	TCCTGGTCCC
OPS- 18	CTGGCGAACT
OPU- 01	ACGGACGTCA

Table 8- PCR conditiones for RAPD reactions

	Temperature ^{o}C	Time	Number of cycles
Initial Denaturation	94	3 min	1
Denaturation	94	1 min	40
Annealing	36	1 min	
Elongation	72	2 min	
Final Elongation	72	10 min	1
The reaction mixture were kept at 4 ^{o}C			

3.2.3. DNA GEL ELECTROPHORESIS AND VISUALIZATION

DNA gel electrophoresis is usually performed for determination of the presence and size of PCR products provided from isolated DNA samples.

In this technique, firstly the agarose solution is boiled until clear. Melted agarose is poured into a form equipped with removable comb. When cooled, the agarose polymerizes, forming a flexible gel. It should appear lighter in color when completely

cooled. Carefully combs are removed from gel and then the gel is placed to electrophoresis chamber. The teeth of removable comb slot in the solidified agarose.

Enough electrophoresis buffer is added to cover the gel to a depth of at least 1 mm. After addition of buffer to the electrophoresis chamber, ethidium bromide can be added to the gel or running buffer either before the gel is run or the gel can be stained after it has run.

DNA samples added looding buffer are placed to the slots and run under the influence of an electrical field through the gel at a neutral pH. Additionally, a DNA ladder is placed to the one of the slots before DNA samples are run. Inclusion of a DNA ladder on the gel makes it easy to determine the sizes of unknown DNAs or PCR products.

DNA is negatively charged due to phosphates in its backbone and for this reason it moves to anode, the positive pole. Small DNA fragments have little frictional drag so move rapidly on the contrary large DNA fragments have more frictional drag so their mobility is slower. Consequently DNA fragments distribute according to their size. While large fragments are near to the top, small fragments are near to the bottom in gel.

In our study, DNA gel electrophoresis is as follows;

1. Gel Loading Buffer (1,5 µl) added to PCR amplification product prior to electrophoresis.
2. About 10 µl from this mixture were loaded to each well.
3. Amplification products were then electrophoresed in 1,4% agarose gels for 1 h and 30 min. at 100 Voltage.

4. Gels were photographed under the ultraviolet light.

3.2.4. EVALUATION OF POPULATIONS WITH NUMERICAL TECHNIQUES

Firstly, the sizes of DNA fragments provided RAPD-PCR for each primer were established in each population separately. Then the tables were constructed containing number and size of the DNA fragments which produced for each populations. Monomorphic and polymorphic bands were determined for all populations. RAPD bands were scored for presence 1 and absence 0.

RAPD data were evaluated by using three different statistical programs. Statistica version 8.0 were used for PCA and CA analysis. Popgen 32 used for genetic similarity, genetic distances, and Amova were used for calculating variations within and among the populations.

3.3. CYTOGENETIC STUDIES

In this part of the study, it was aimed that determination of variations between populations depending on chromosomes. For this aim, a few population belonging to three species were karyotyped. Fruits of plant samples were collected during 2008–2009 in different areas of Turkey (Table 2, 3 and 4).

Firstly, fruits of these samples were germinated. Different techniques have been used for germination. For example, acorns of these samples were placed into plastic cups filled with water and kept at room temperature. This technique was successful in *Q.coccifera* but it was not successful for the remained species. *Q. ilex* and *Q. aucheri* fruits were placed into nylon bag and kept in refrigerator. At intervals, fruits within nylon bag were washed for the moisture.

As a first treatment; α-monobromonaphthalene was used for 16–17 hours at 4°C. Afterwards; 3:1 alcohol-glacial acetic acid mixture was used for overnight as fixative. Prior to staining, hydrolysis was done in 1N HCl at 60°C for 30 minutes. Then root tips were stained with feulgen for 2 hours and squashes were made with 2% aceto-orcein. The preparations were frozen in liquid nitrogen and made permanent with Entellan. The photographs enlarged on 10x100 were taken using Olympus BX51 microscope with camera DP71 attachment.

CHAPTER IV

4. RESULTS

4.1. MORPHOLOGICAL STUDIES

Numerical results of leaf and fruit were evaluated separately. After that, these results were compared with each other.

4.1.1. MORPHOLOGICAL STUDIES ON LEAVES

When the results provided from CA are evaluated, two main groups are observed. One of these groups consists of populations belonging to *Q. ilex*. Populations belonging to *Q. coccifera* and *Q. aucheri* take place in the second group. In other words, it can be stated that populations of *Q. ilex* show more differences than populations of *Q. coccifera* and *Q. aucheri*. Populations belonging to *Q. ilex* are placed geographically close to each other and showed more similarity than others. According to the results of CA, first group containing populations of *Q. ilex* is divided into two sub-groups. One of these consists of I1, I2 and I3 populations. Other sub-group consists of I4 and I5 populations. Populations of I1 and I2 are the two closely located and had the highest similarity. The I3 population is geographically close to I1 and I2 populations. Localities of the second sub-group populations are away from other three populations. When the other taxa populations are examined except C7 population, it is observed that the second main group is divided into two sub-groups containing all populations of *Q. coccifera*

and *Q. aucheri*. One of these sub-groups consists of all populations of *Q. aucheri* and only two populations of *Q. coccifera*. These two populations of *Q. coccifera* are C17 and C5. The last sub-group consists of completely populations of *Q. coccifera* (Figure 9). Highest differences within this sub-group are observed in C14, C15 and C16 populations.

On the other hand, PCA analysis clearly separates the *Q. ilex* populations. *Q. coccifera* and *Q. aucheri* showed more similarity. Therefore; two main groups are observed according to PCA results. One of these groups consists of populations belonging to *Q. ilex*. Populations of other two taxa are evaluated in the second group. In this group, populations of *Q. coccifera* showed differences from each other. Populations showing the most differences in *Q. coccifera* are C17, C16 and C15 (Figure 10). When the PCA results are compared with CA, high similarity can be recognized. But PCA analysis could not separate clearly the taxa like CA.

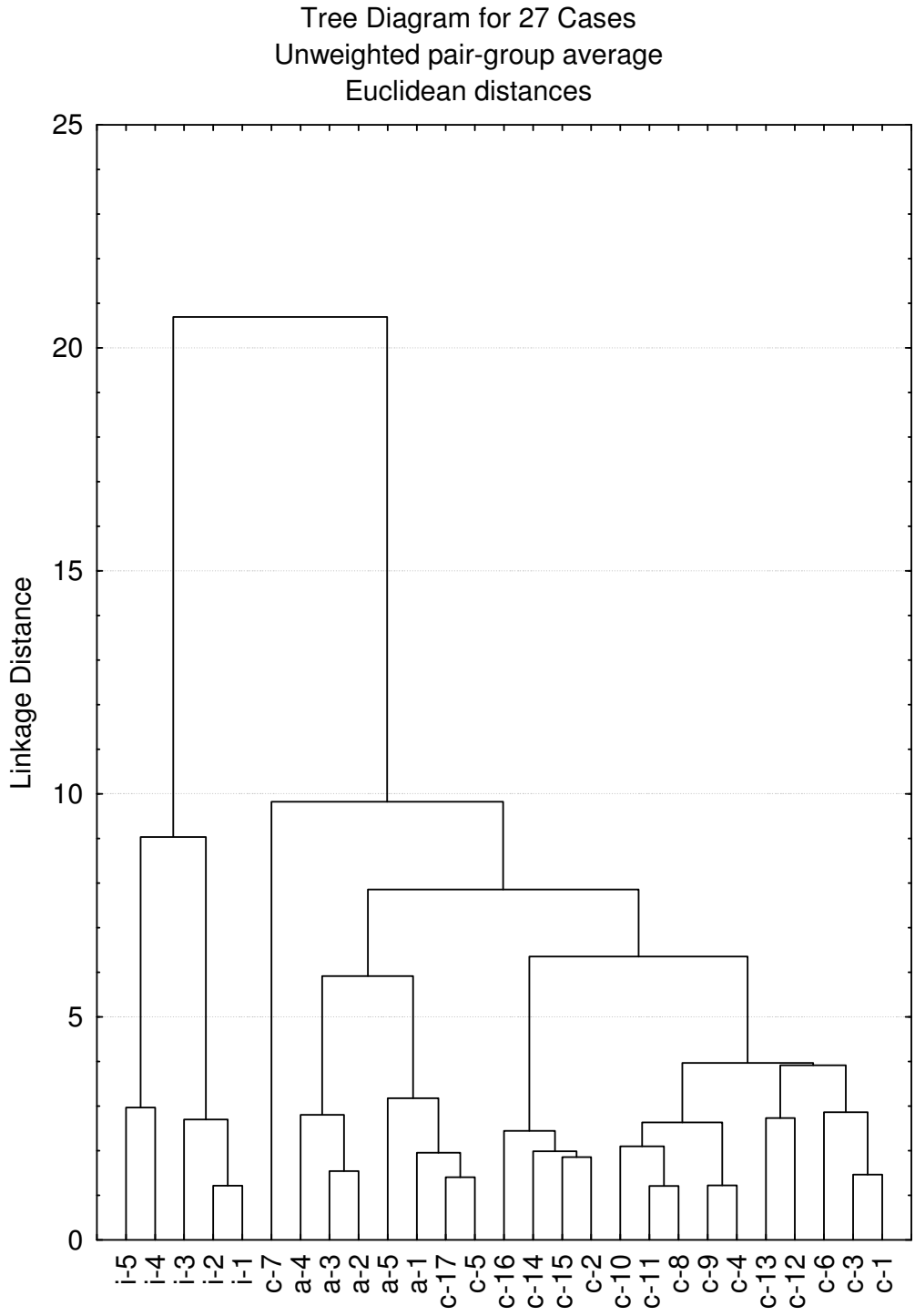

Figure 9- Phenogram resulting from Cluster Analyses with UPGMA for leaf characters

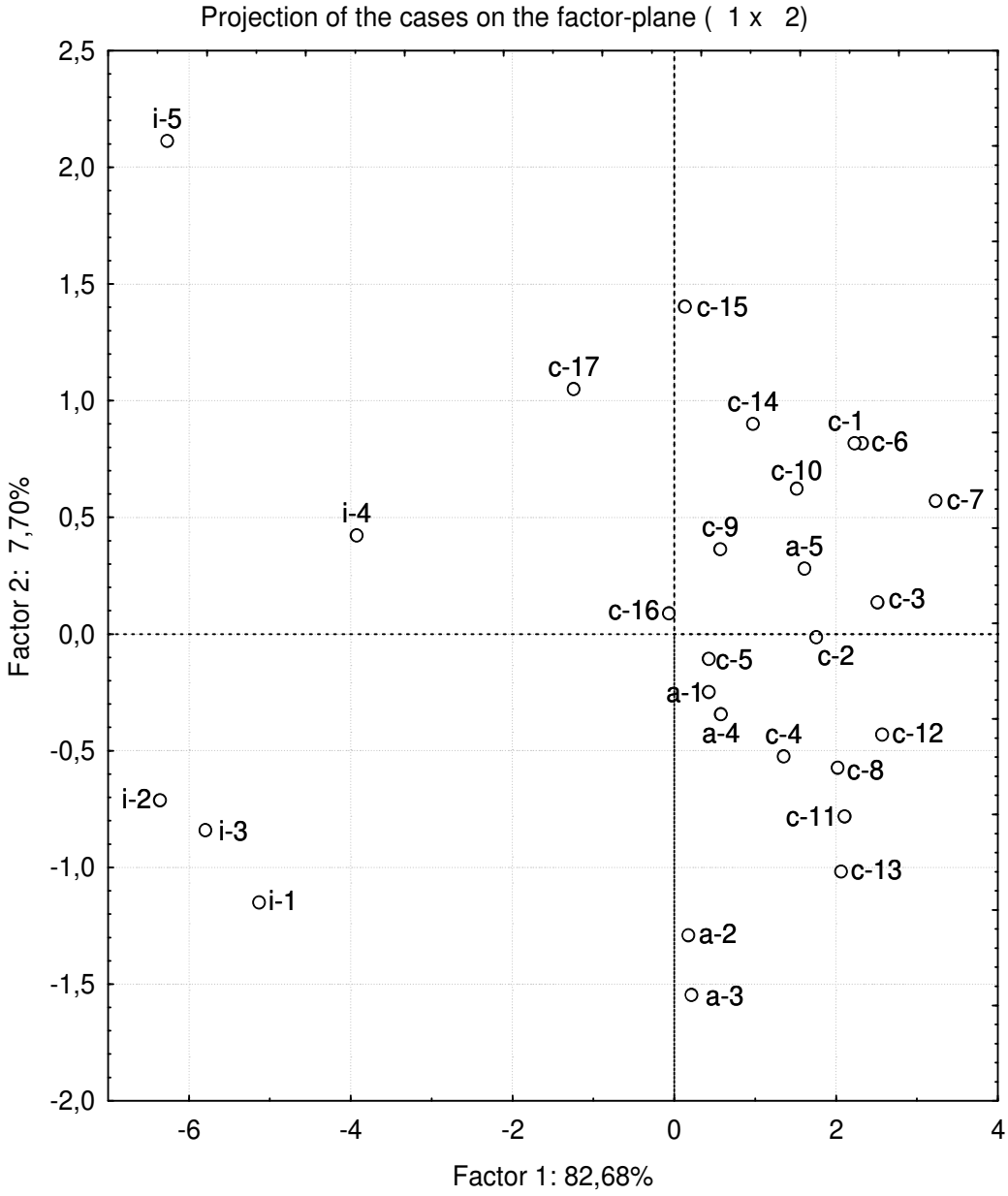

Figure 10- The result of Principal Component Analyses for leaf characters

4.1.2. MORPHOLOGICAL STUDIES ON FRUITS

In general the results of leaves and fruits are showed similarity. According to the CA results, there are two main groups for fruits as well (Figure 11). First main group consists of populations of *Q. ilex* and the second main group has the populations of *Q.*

coccifera and *Q. aucheri*. Second group is divided into two sub-groups. One of these groups includes all populations of *Q. aucheri* and the 5 populations of *Q. coccifera* known as C10, C14, C15, C16 and C17. Other group consists of the remaining 12 populations of *Q. coccifera*.

Likewise, similar results are observed in PCA with fruits (Figure 12-13). The y-axis (component of I: 44.54%) clearly separates the *Q. coccifera* populations in two groups (Figure 13). Additionally, the populations of *Q. coccifera* on East Mediterranean region are appeared among the group of *Q. aucheri* populations.

Especially, when the Figure 13 the results of PCA is investigated carefully, differences among species are clearly observed. According to Figure 13, populations of *Q. ilex* show the highest variations and a group of *Q. coccifera* (C14, C15, C16 and C17) from East Mediterranean region is clustered more or less under the same group with *Q. aucheri*.

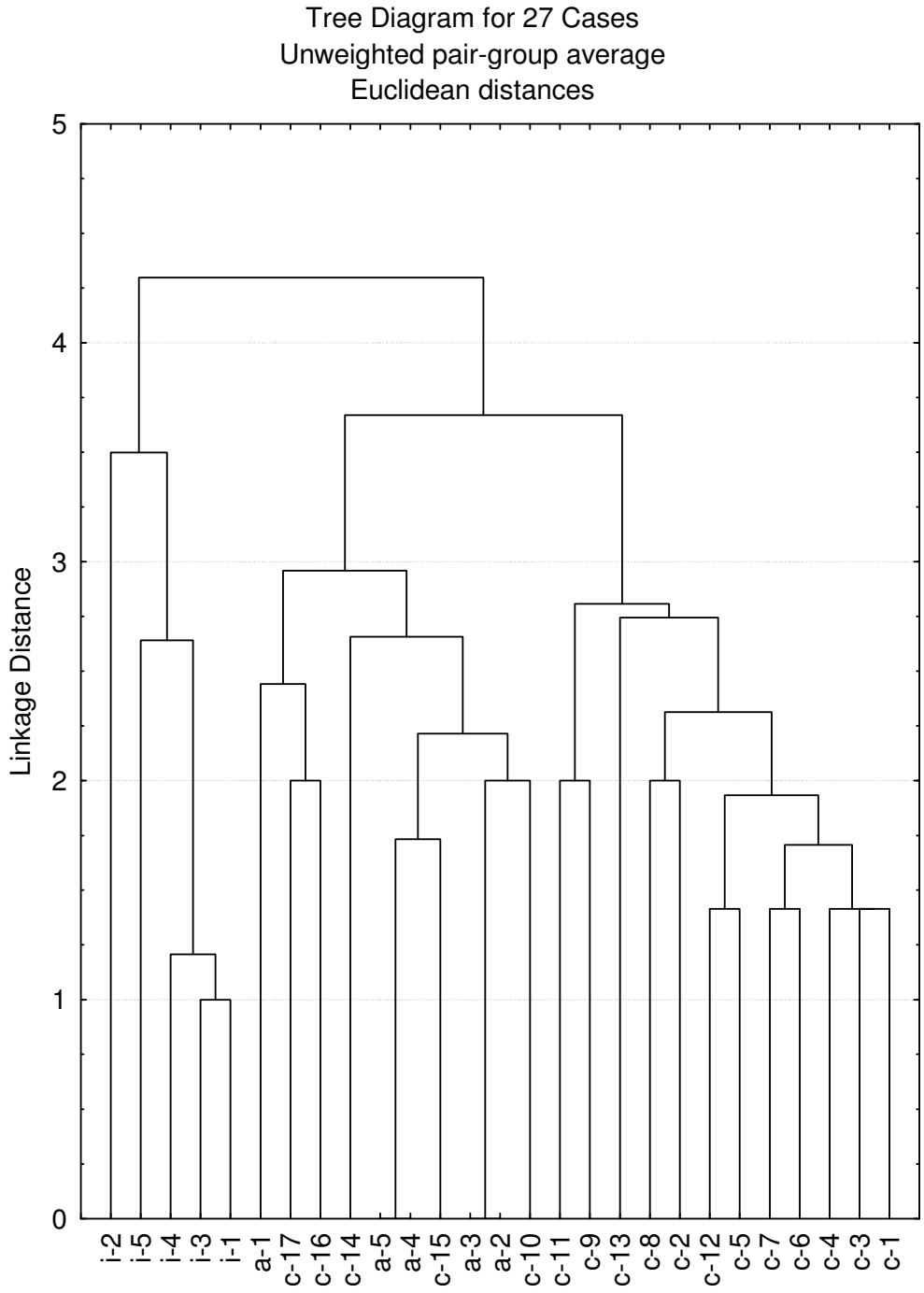

Figure 11- Phenogram resulting from Cluster Analyses with UPGMA for fruit characters

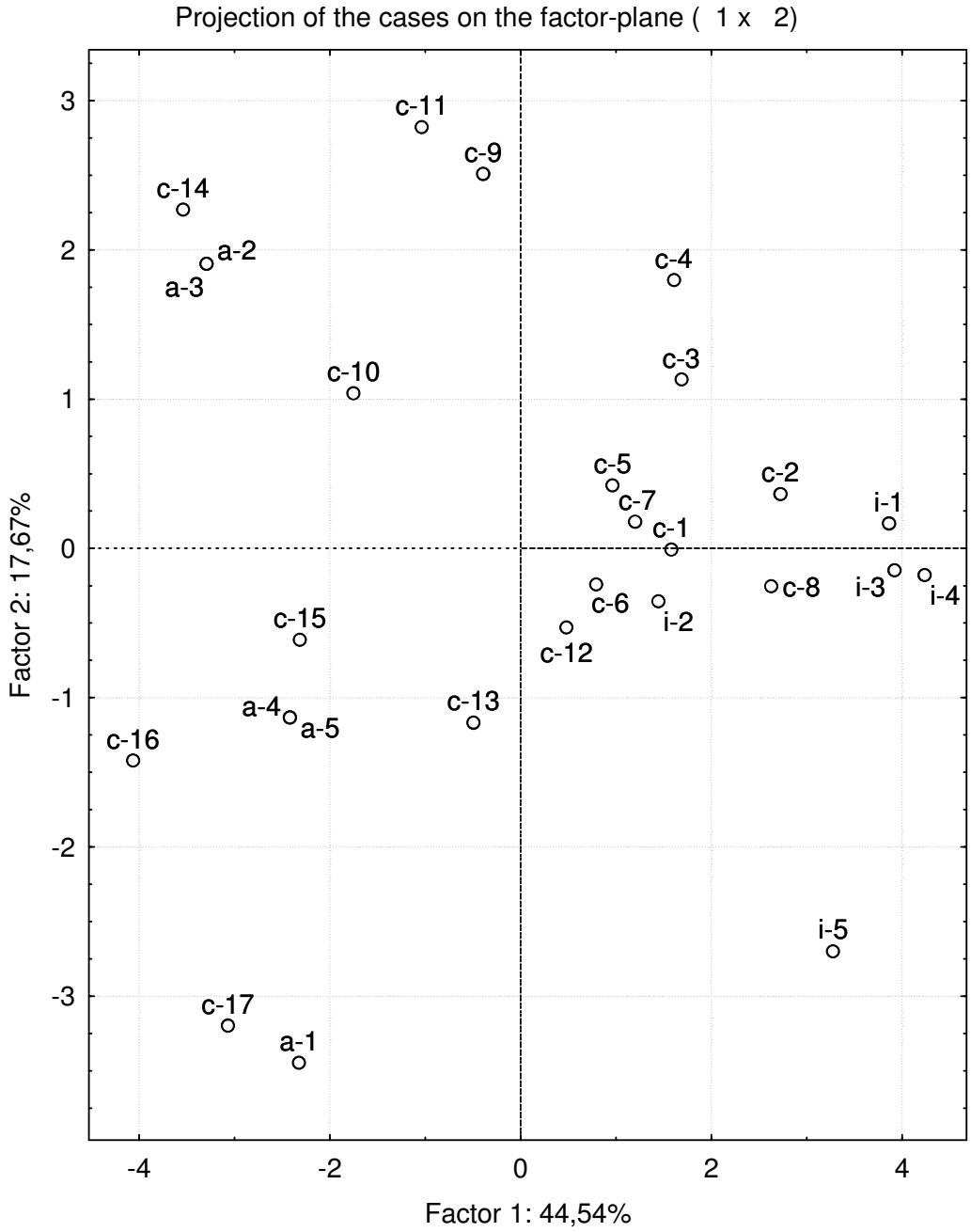

Figure 12- The result of Principal Component Analyses for fruit characters with Factor1-2

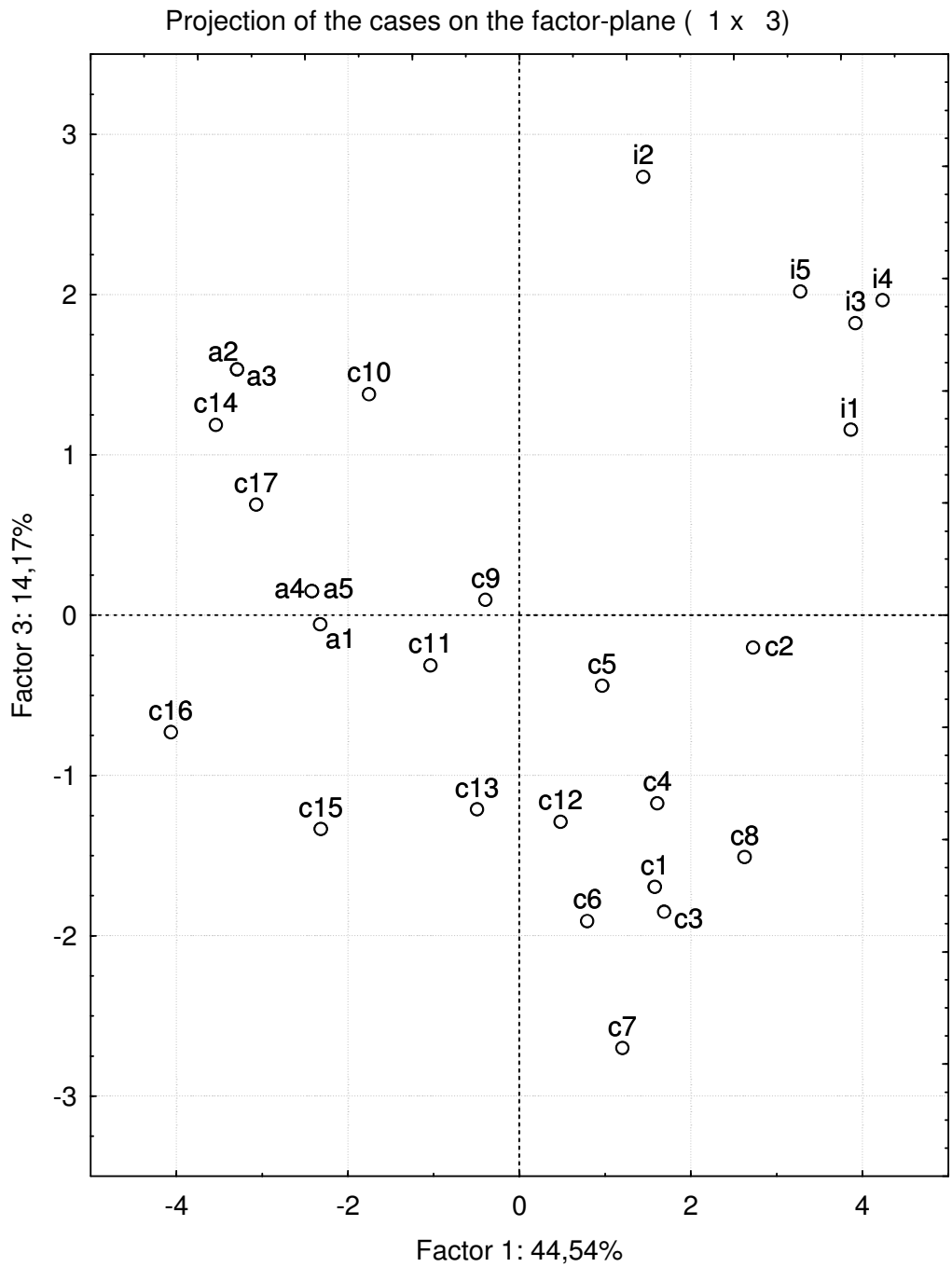

Figure 13- The result of Principal Component Analyses for fruit characters with Factor 1-3

4.2. MOLECULAR STUDIES

In the RAPD analysis, 162 individuals (samples) representing 27 populations were used. A total 219 polymorphic bands were scored using the 10 RAPD primers. The size of the amplication products was between 150 to1600 base-pair. Table 9 shows the total number of polymorphic bands provided from each primers. The minimum and maximum size of amplification products provided from different primers were also listed in Table 10.

Table 9- The total number of polymorphic bands provided from each primers

PRIMERS (bp)	**Sequence (5^{l}-3^{l})**	**Number of bands**
OPA-01	CAGGCCCTTC	20
OPA-08	GTGACGTAGG	18
OPA-09	GGGTAACGCC	21
OPB-04	GGACTGGAGT	22
OPX-04	CCGCTACCGA	23
OPC-03	GGGGGTCTTT	23
OPC-09	CTCACCGTCC	19
OPS-09	TCCTGGTCCC	25
OPS-18	CTGGCGAACT	24
OPU-01	ACGGACGTCA	24

Table 10- The minimum and maximum size of amplification products provided from different primers in studied populations

	PRIMERS products sizes (min basepair-max basepair)									
Population	**OPA-01**	**OPA-08**	**OPA-09**	**OPB-04**	**OPC-09**	**OPS-09**	**OPS-18**	**OPU-01**	**OPX-04**	**OPC-03**
c_1	400-1200	250-800	350-1400	200-1100	400-1250	400-1400	200-1000	200-800	200-1200	400-1300
c_2	400-1200	300-800	350-1200	600-1100	300-1150	300-1300	350-1300	250-1200	250-1200	400-1300
c_3	500-1150	300-900	400-1250	450-1150	400-1100	300-1100	200-600	350-1200	200-1200	350-1300
c_4	400-1200	300-900	350-1250	450-1100	450-1300	200-1400	350-1100	300-1000	250-1300	400-1300
c_5	400-1200	200-800	350-1150	350-1100	300-1050	200-1200	300-1000	200-1150	150-1200	450-1250
c_6	300-1100	200-1100	300-1400	550-1100	500-1100	300-1400	550-1150	350-1150	200-1300	400-1300
c_7	500-1150	200-1100	350-1400	200-1100	500-1250	300-1300	200-800	300-1100	150-1000	400-1300
c_8	400-1200	200-800	300-1000	400-1150	400-1250	500-1050	300-1100	200-1000	200-1100	400-1300
c_9	500-1150	200-700	300-1400	550-1050	500-1250	250-1350	350-1100	300-900	400-1400	400-1300
c_{10}	400-1150	300-700	300-1400	600-1150	300-1250	300-1350	200-1100	300-1100	150-1200	400-1300
c_{11}	400-1200	200-1100	300-1200	500-1050	500-1250	300-1250	200-1100	300-1200	150-650	400-1300
c_{12}	400-1200	200-1100	350-1250	600-1050	500-1250	600-1250	200-1300	300-1150	150-1100	400-1300
c_{13}	400-1200	200-900	300-1400	600-1050	500-1250	600-1350	350-1050	200-1150	150-1100	400-1300
c_{14}	500-1200	200-1200	350-1250	400-1200	500-1250	300-1300	350-1250	300-1150	300-1400	300-1300
c_{15}	500-1150	300-800	300-1400	200-1200	500-1250	300-1350	200-1100	250-1200	650-1400	600-1200
c_{16}	400-700	200-1050	350-1400	200-1200	500-1250	300-1300	200-1000	300-1150	600-1400	550-1100
c_{17}	500-1150	200-1050	300-1400	650-1150	500-1250	500-1350	550-1200	300-1200	300-1100	550-1300
i_1	400-1200	300-1200	300-1400	200-1400	700-1250	500-1400	300-1100	200-1300	400-1000	400-1300
i_2	600-1250	300-1200	400-1200	300-1300	700-1250	500-1400	300-1300	400-1250	300-1150	700-1300
i_3	400-1250	300-1200	400-1200	300-1400	700-1250	300-1500	200-800	400-1400	200-1100	400-1300
i_4	500-1250	200-1100	400-1200	500-1400	500-1250	300-1300	200-1000	650-1300	200-1100	600-1300
i_5	400-1350	200-1100	400-1200	500-1100	500-1250	500-1300	200-1150	400-1250	200-1100	400-1300
a_1	500-1250	300-700	250-900	200-1100	300-1250	300-1200	200-900	300-1100	200-1100	400-1500
a_2	300-1400	300-700	400-900	300-1100	500-1250	200-1350	200-1000	200-1000	200-1000	150-1300
a_3	300-1400	200-1400	300-1400	200-1100	300-1200	400-1400	200-1000	200-1000	150-650	400-1500
a_4	500-1400	300-1000	300-900	200-1200	500-1250	400-1500	200-1400	200-1000	150-1400	400-1600
a_5	500-1400	300-800	300-1200	200-1100	600-1250	350-1500	200-1000	300-1200	150-1400	400-1400

In order to score the RAPD products, individuals of each population were run separately for each primer. Some examples of these RAPD products were given in Figure 14, 15, 16, 17 and 18. Additionally, six individuals of RAPD products from every population were bulked and run together, to see all populations products in the same gel for each primer. Some examples of these gels were also presented in Figure 19, 20, 21 and 22.

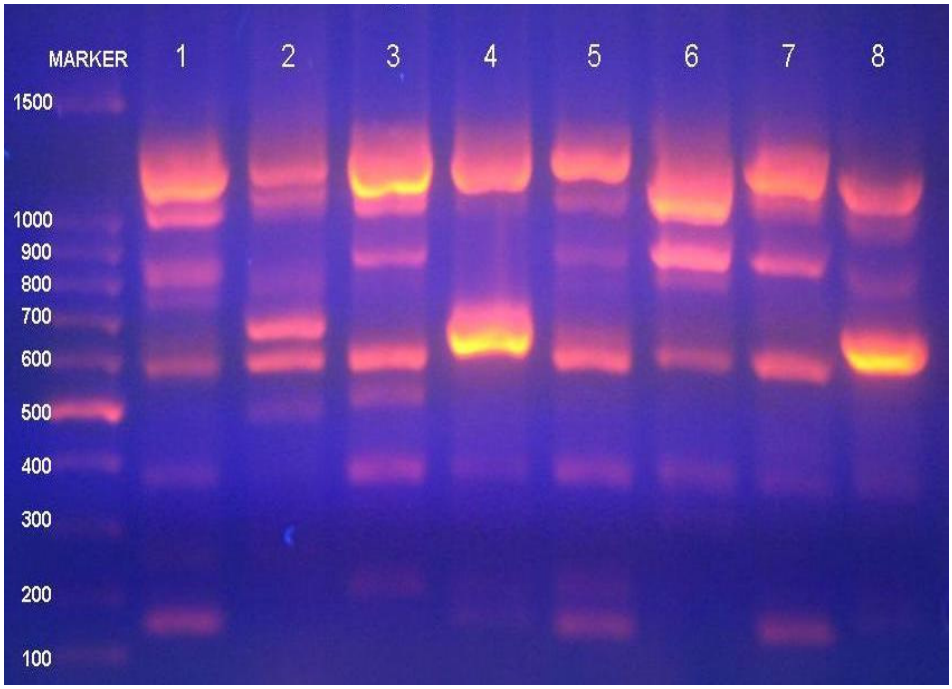

Figure 14- RAPD products in C13 population with OPX-04 primer

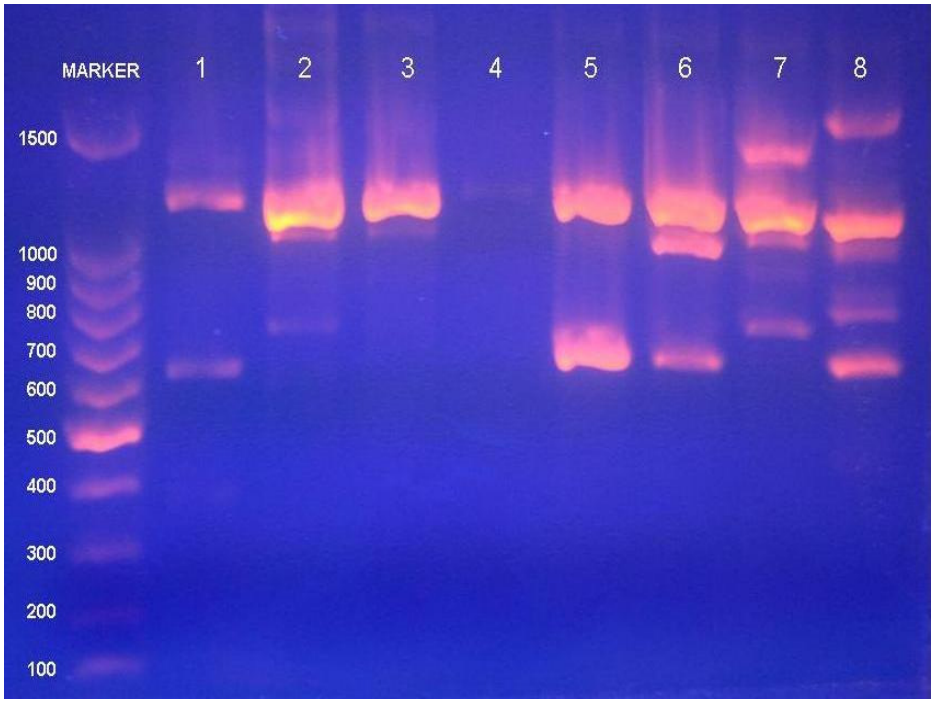

Figure 15- RAPD products in C15 population with OPX-04 primer

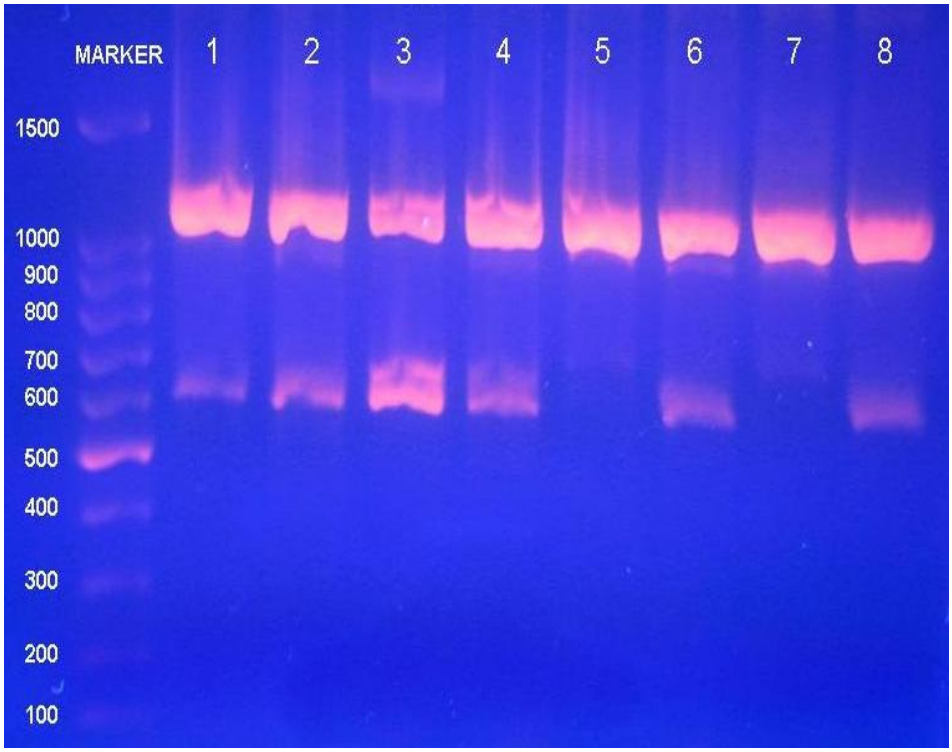

Figure 16- RAPD products in C16 population with OPX-04 primer

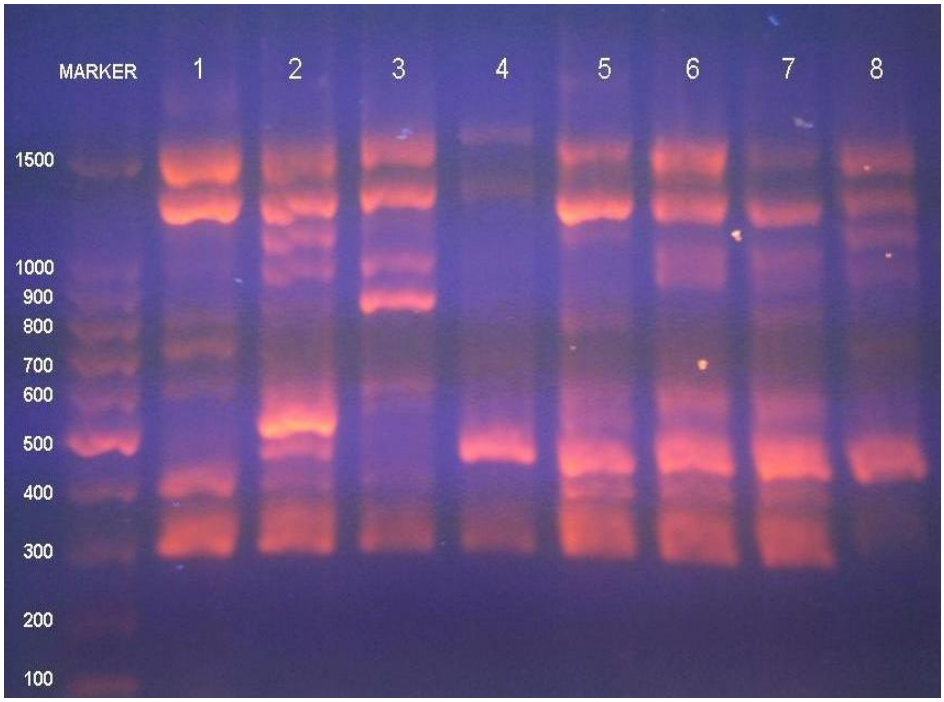

Figure 17- RAPD products in C15 population with OPA-09 primer

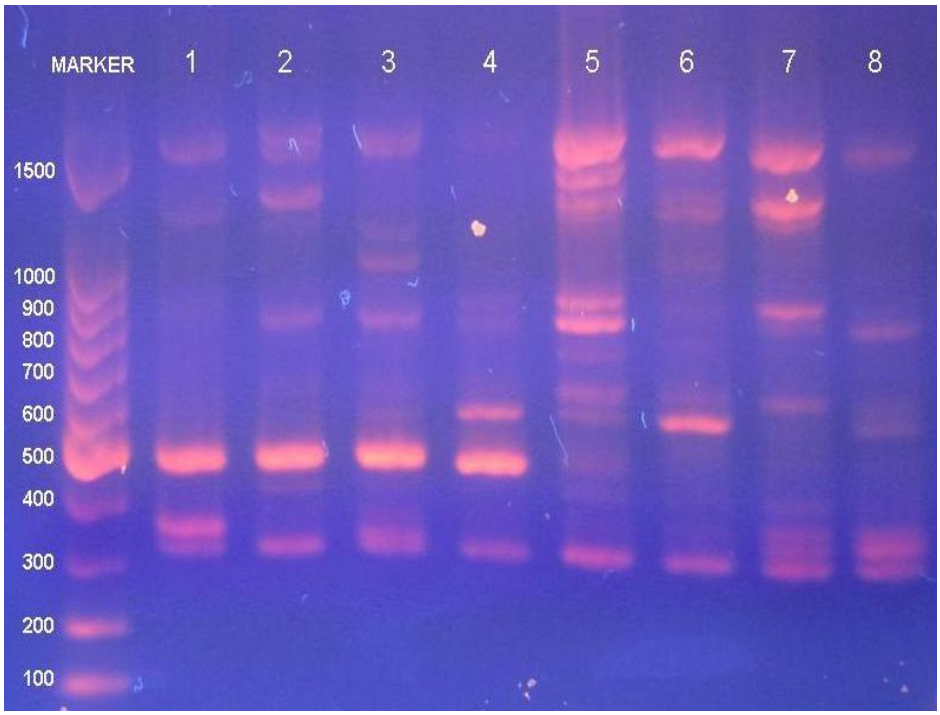

Figure 18- RAPD products in C16 population with OPA-09 primer

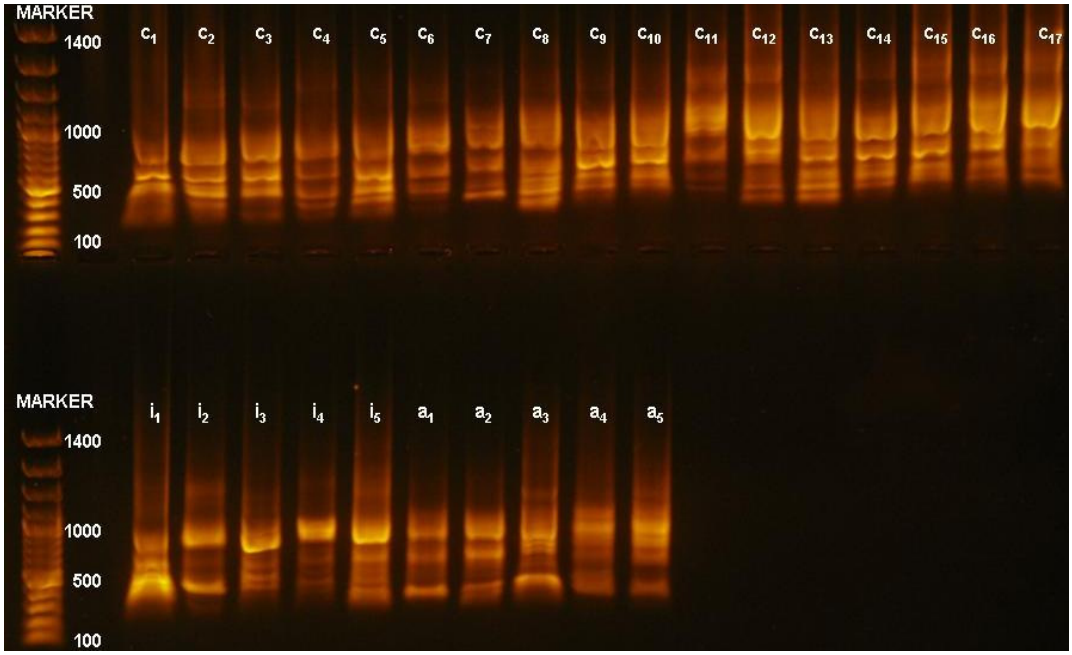

Figure 19- Visualization of all population's RAPD products with OPU-01 primer

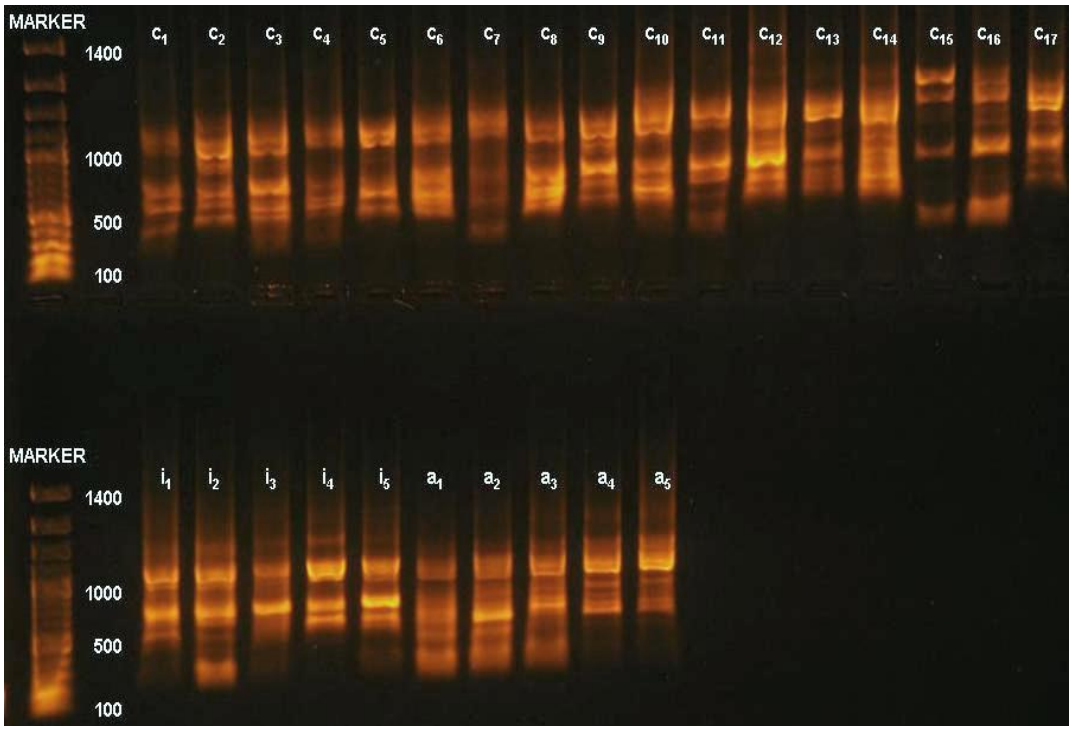

Figure 20- Visualization of all population's RAPD products with OPB-04 primer

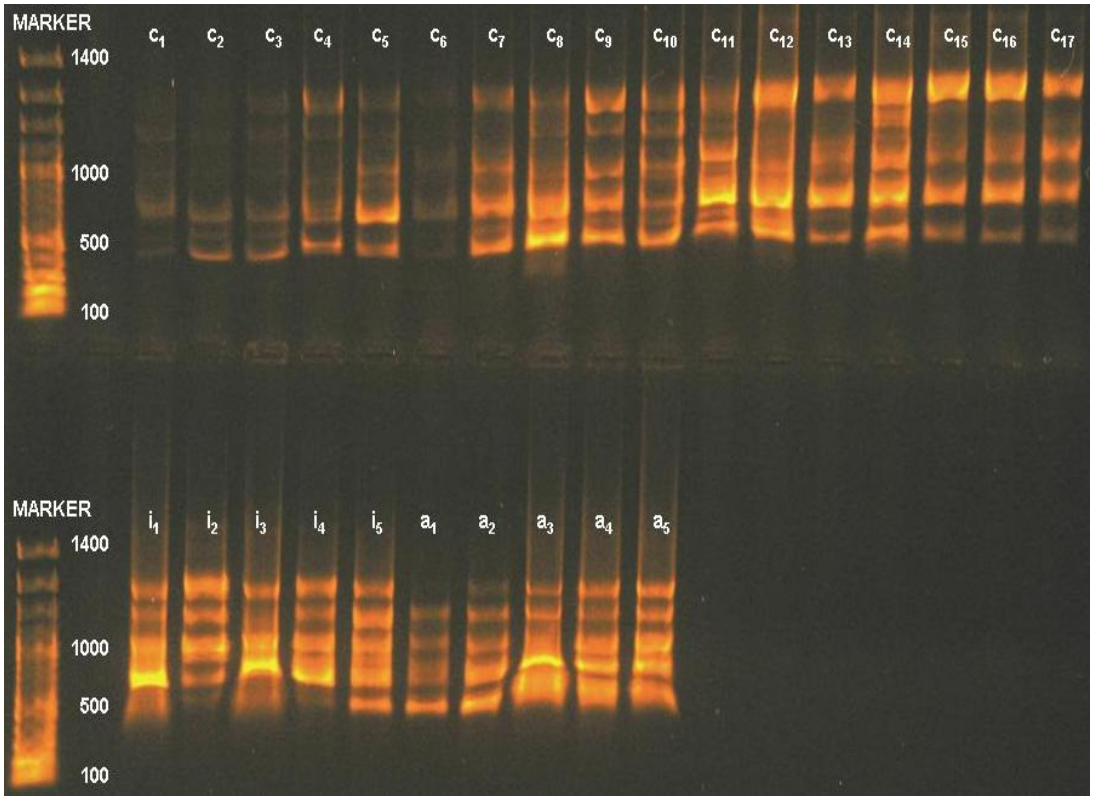

Figure 21- Visualization of all population's RAPD products with OPC-03 primer

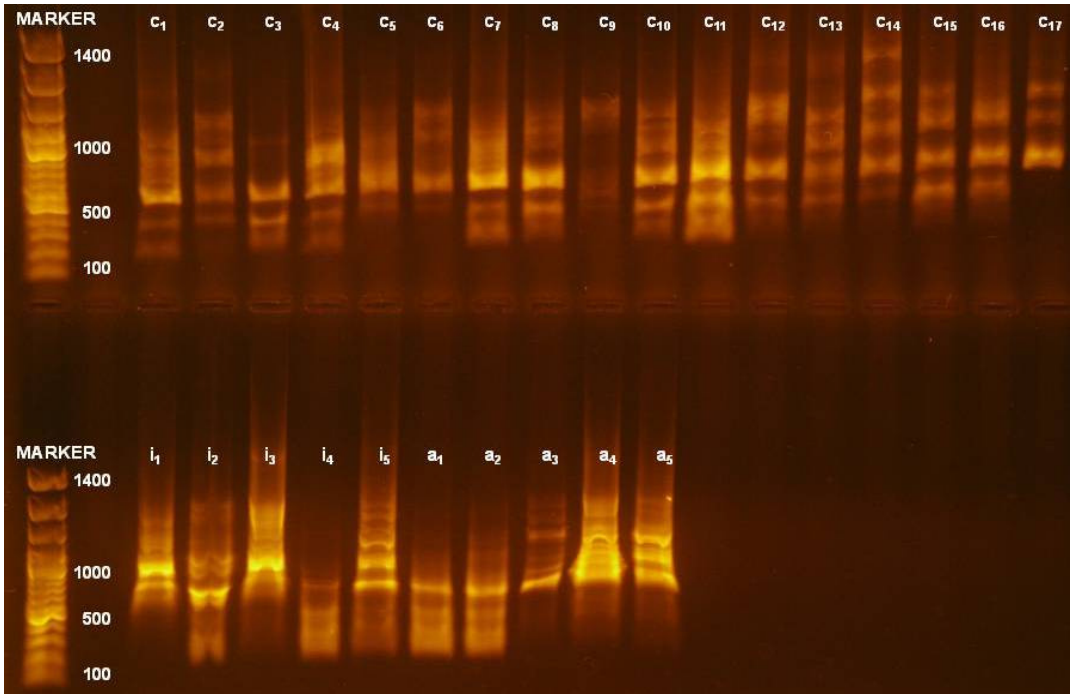

Figure 22- Visualization of all population's RAPD products with OPS-18 primer

4.2.1. CA AND PCA ANALYSES

In order to compare the results of morphological with molecular, CA and PCA analyses were also made with molecular results. According to these results, *Q. ilex* and *Q. aucheri* is observed as close two separate groups. Populations of *Q. coccifera* show more differences than populations of *Q. ilex* and *Q. aucheri*. But fundamentally, three studied species show differences from each other.

When the each species is evaluated separately, generally geographically close populations show more similarity than geographically distant ones (Figure 23). Populations belonging to *Q. ilex* are separated into two sub-groups in CA pehenogram. The first of these are I1, I2 and I3 populations. The second sub-group of *Q. ilex* are composed of I4 and I5 populations. Other species, *Q. aucheri* is separated into two sub-groups like *Q. ilex* but here C7 population of *Q. coccifera* shows the high similarity with the populations of *Q. aucheri*. The same result is observed in the phenogram of leaf characters. Finally, when the populations of *Q. coccifera* are examined, it draws attention that *Q. coccifera* is separated into three sub-groups. When these three sub-groups are observed attentively, they are separated as geographically from each other (Figure 23).

Populations belonging to *Q. coccifera* evaluated in the Southern Marmara and Northern Aegean Region are C1, C2, C3, C4, C5 and C6 according to the phenogram in Figure 23 resulting from Cluster analysis with UPGMA. Only C6 is the population located on the Mediterranean region as distinct from the others. Populations of *Q. coccifera* evaluated in the Southern Aegean and Western Mediterranean region are C8, C9, C10, C11, C12 and C13.

Eventually, populations having the highest differences belonging to *Q. coccifera* are C14, C15, C16 and C17. These populations except C14, are originated from East Mediterranean region (Figure 23-24). Similar findings were observed with morphological results.

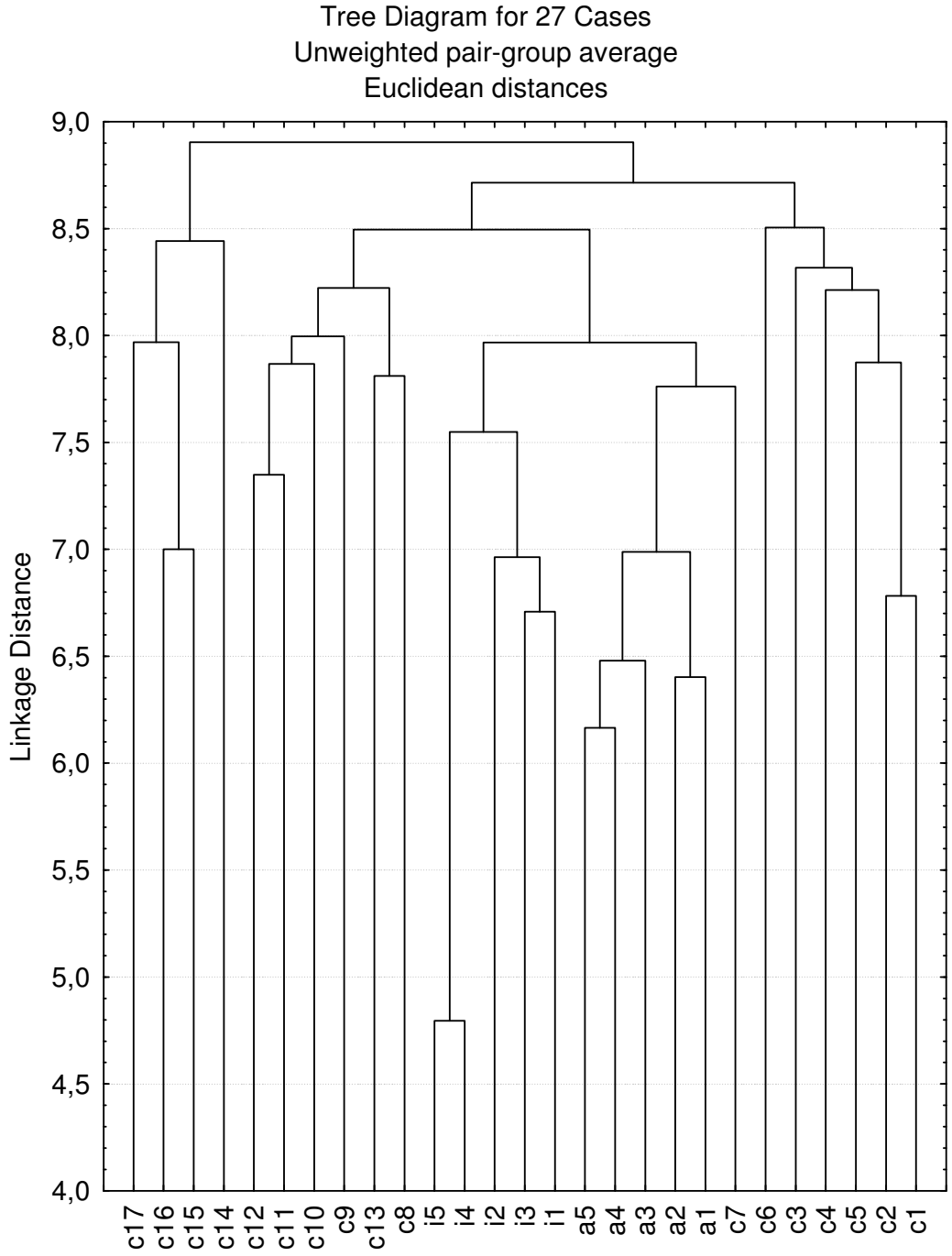

Figure 23- Phenogram resulting from Cluster Analysis with UPGMA for molecular study

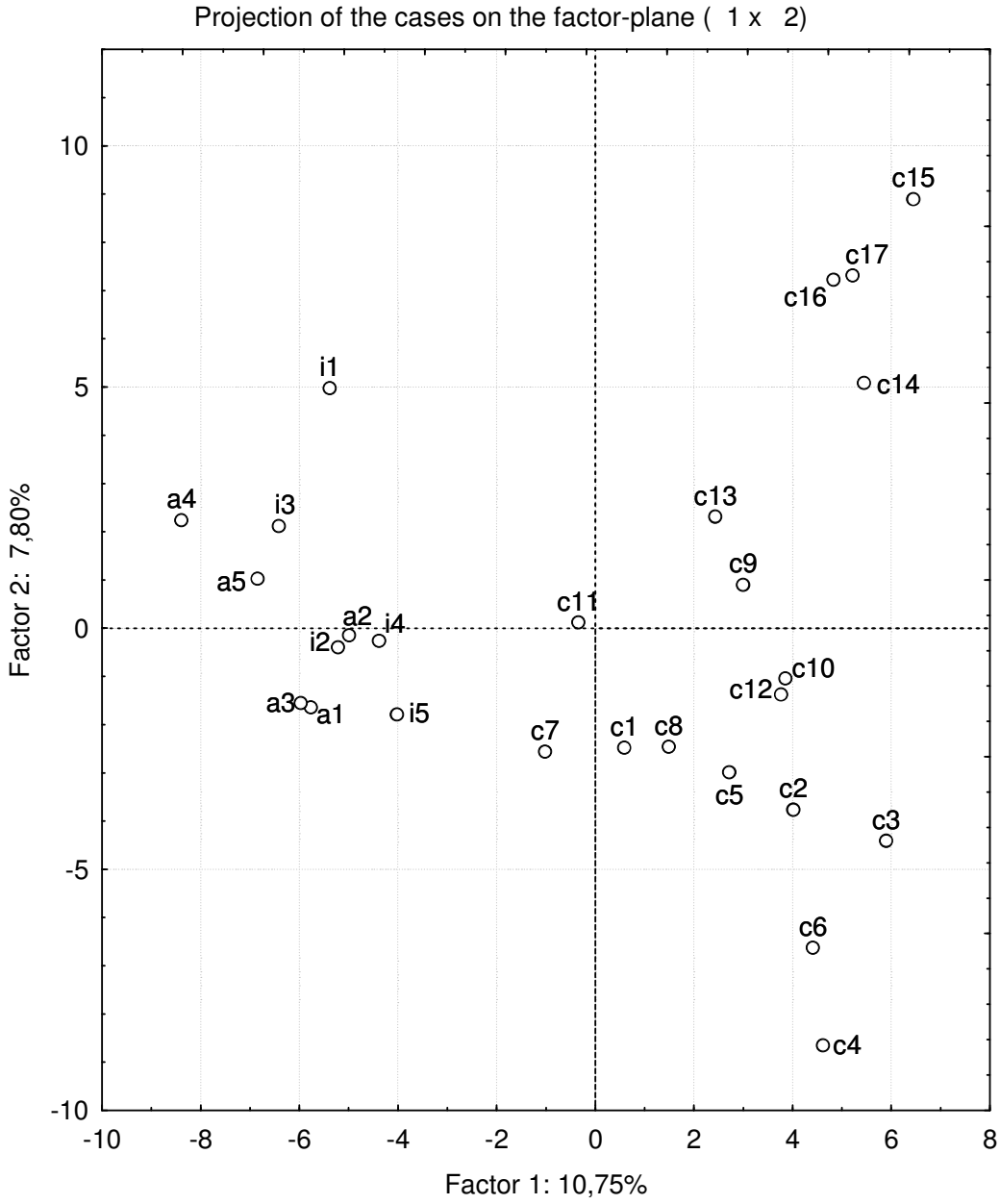

Figure 24- The result of Principal Component Analysis for molecular study

4.2.2. GENETIC VARIATION AND GENETIC DISTANCE

When the populations of *Q. coccifera* are evaluated for genetic variation in Table 11, C14 (Yalova) population has the highest value with H= 0,417. The second high variation value with H= 0,404 was found in C4 population. C3 population has the

lowest value with H= 0,336. Among the *Q. ilex* populations I1 population shows the highest genetic variation, I2 population has the lowest variation with H= 0,340. Among the populations of *Q. aucheri*, A4 population has the highest value of genetic variation with H= 0,401. Population A2 has the lowest value with H= 0,369.

In general, when we make the comparison among the all studied species populations; the mean of *Q. aucheri* populations has the highest variation (H=0,382), the second variation has among the populations of *Q. ilex* (H=0,379) and the least variation has among the *Q. coccifera* populations (H=0,371) (Table 11).

The total gene diversity for the populations provided from section *Ilex* of the genus *Quercus* is Ht= 0,383. Genetic differentiation value among studied populations (Gst) and gene flow (Nm) is 0,020 and 23,552 respectively (Table 13).

Mean number of effective alleles (Ne) was always lower than Na as expected. The highest Ne value with 1,730 was observed in C14 population, whereas the lowest Ne value with 1,507 was found in C3 population. The Ne value of *Q. ilex* populations and *Q. aucheri* populations were found in between those *Q. coccifera* values. According to Shannon index (I) variations level *Q. aucheri* populations were first then *Q. ilex* were second and finally *Q. coccifera* populations showed the least variations (Table 11).

When the tables of genetic distance and similarity is investigated, Table 14, the lowest genetic distance is observed between C1-C2 and A1-A5 populations. In other words, the highest genetic similarity is observed between C1-C2 and A1-A5 populations. The highest genetic distance is between C15-A4 and C15-A3 populations, respectively. In other words, the lowest genetic similarity is between C15-A4 and C15-

A3 populations.

The AMOVA performed over 27 populations to find out variations among and within the populations. In Table 12, variation was found high among the populations 71,59 % . On the contrary variation was less within populations 28,41 %.

Table 11- Genetic variation analyses for all locus

Population	*Na	*Ne	*H	*I
c_1	2,000	1,571	0,357	0,540
c_2	2,000	1,544	0,342	0,521
c_3	2,000	1,507	0,336	0,519
c_4	2,000	1,697	0,404	0,592
c_5	2,000	1,641	0,383	0,569
c_6	2,000	1,578	0,354	0,536
c_7	2,000	1,606	0,373	0,558
c_8	2,000	1,634	0,381	0,568
c_9	2,000	1,638	0,384	0,571
c_{10}	2,000	1,672	0,396	0,584
c_{11}	2,000	1,597	0,369	0,555
c_{12}	2,000	1,626	0,377	0,561
c_{13}	2,000	1,658	0,389	0,576
c_{14}	2,000	1,730	0,417	0,607
c_{15}	2,000	1,603	0,361	0,542
c_{16}	2,000	1,537	0,338	0,518
c_{17}	2,000	1,594	0,361	0,543
Mean of *Q. coccifera*'s	2,000	**1,613**	**0,371**	**0,556**
i_1	2,000	1,708	0,411	0,601
i_2	2,000	1,525	0,340	0,522
i_3	2,000	1,672	0,396	0,584
i_4	2,000	1,615	0,377	0,563
i_5	2,000	1,606	0,374	0,561
Mean of *Q. ilex*'s	2,000	**1,625**	**0,379**	**0,566**
a_1	2,000	1,649	0,387	0,574
a_2	2,000	1,605	0,369	0,553
a_3	2,000	1,614	0,376	0,562
a_4	2,000	1,689	0,401	0,589
a_5	2,000	1,623	0,379	0,566
Mean of *Q. aucheri*'s	2,000	**1,636**	**0,382**	**0,568**
General Mean	2,000	**1,627**	**0,383**	**0,571**

*Na: Expected alleles

*Ne: Effective alleles

*H: Genetic variation

*I: Shannon index

Table 12- Results from analyses of molecular variance (AMOVA) based on 162 individuals from 27 populations

Source of variation	d.f.*	Sum of squares	Variance	Percentage (%)
Among population	26	3846,333	23,126	71,59
Within population	135	1238,833	9,176	28,41
Total	161	5085,166	32,302	100
*d.f. : Degrees of Freedom				

Table 13- Genetic diversity analyses for all locus (Nei, 1977)

Population number	Ht	Hs	Gst	Nm
27	0,383	0,375	0,020	23,552

Ht: Total gene diversity

Hs: Gene diversity within populations

Gst: Genetic differentiation

Nm: Gene flow

Table 14- The comparison of genetic distance (below diagonal) and genetic similarity (upper diagonal) (Nei, 1972)

	c_1	c_2	c_3	c_4	c_5	c_6	c_7	c_8	c_9	c_{10}	c_{11}	c_{12}	c_{13}	c_{14}	c_{15}	c_{16}	c_{17}	i_1	i_2	i_3	i_4	i_5	a_1	a_2	a_3	a_4	a_5
c_1	****	**0.998**	0.985	0.991	0.991	0.992	0.993	0.991	0.996	0.991	0.992	0.988	0.990	0.986	0.985	0.988	0.986	0.985	0.990	0.987	0.991	0.995	0.995	0.994	0.990	0.990	0.996
c_2	**0.001**	****	0.981	0.990	0.989	0.992	0.990	0.987	0.993	0.987	0.991	0.985	0.987	0.984	0.982	0.985	0.983	0.981	0.986	0.982	0.986	0.992	0.991	0.992	0.986	0.987	0.991
c_3	0.014	0.018	****	0.971	0.973	0.987	0.986	0.987	0.980	0.987	0.990	0.984	0.982	0.969	0.973	0.990	0.989	0.976	0.986	0.987	0.985	0.988	0.979	0.986	0.989	0.973	0.981
c_4	0.008	0.010	0.029	****	0.985	0.990	0.989	0.983	0.994	0.985	0.980	0.978	0.982	0.990	0.974	0.973	0.977	0.981	0.977	0.984	0.985	0.987	0.992	0.985	0.981	0.993	0.991
c_5	0.009	0.010	0.026	0.014	****	0.987	0.990	0.992	0.989	0.987	0.989	0.995	0.993	0.984	0.976	0.981	0.981	0.981	0.980	0.985	0.989	0.991	0.991	0.989	0.986	0.989	0.992
c_6	0.007	0.007	0.012	0.009	0.013	****	0.990	0.987	0.992	0.987	0.988	0.985	0.987	0.979	0.977	0.986	0.986	0.977	0.979	0.985	0.986	0.988	0.986	0.988	0.982	0.985	0.988
c_7	0.006	0.009	0.013	0.011	0.009	0.009	****	0.993	0.991	0.989	0.990	0.990	0.990	0.990	0.981	0.990	0.990	0.986	0.992	0.989	0.992	0.992	0.994	0.990	0.991	0.989	0.993
c_8	0.008	0.012	0.012	0.016	0.007	0.012	0.006	****	0.986	0.991	0.990	0.997	0.994	0.983	0.977	0.987	0.989	0.982	0.984	0.992	0.989	0.994	0.994	0.993	0.994	0.987	0.991
c_9	0.003	0.006	0.019	0.005	0.011	0.007	0.008	0.013	****	0.992	0.990	0.983	0.989	0.986	0.985	0.987	0.983	0.985	0.988	0.987	0.992	0.992	0.994	0.992	0.983	0.992	0.996
c_{10}	0.008	0.012	0.013	0.015	0.012	0.012	0.011	0.008	0.007	****	0.992	0.990	0.996	0.985	0.989	0.992	0.991	0.988	0.983	0.985	0.992	0.991	0.989	0.989	0.987	0.983	0.989
c_{11}	0.007	0.008	0.009	0.020	0.010	0.011	0.009	0.009	0.010	0.007	****	0.990	0.990	0.983	0.984	0.991	0.988	0.990	0.990	0.988	0.989	0.993	0.987	0.991	0.990	0.982	0.989
c_{12}	0.011	0.014	0.015	0.021	0.004	0.014	0.009	0.002	0.016	0.009	0.009	****	0.995	0.980	0.972	0.983	0.986	0.979	0.981	0.988	0.991	0.993	0.989	0.990	0.993	0.985	0.989
c_{13}	0.009	0.013	0.017	0.017	0.006	0.012	0.010	0.005	0.011	0.003	0.009	0.004	****	0.986	0.987	0.989	0.993	0.987	0.977	0.983	0.991	0.989	0.988	0.986	0.986	0.981	0.988
c_{14}	0.013	0.016	0.031	0.009	0.015	0.020	0.009	0.016	0.013	0.014	0.016	0.020	0.013	****	0.980	0.976	0.984	0.993	0.977	0.978	0.984	0.983	0.986	0.977	0.983	0.983	0.983
c_{15}	0.014	0.017	0.026	0.026	0.024	0.023	0.019	0.022	0.014	0.010	0.015	0.028	0.012	0.019	****	0.992	0.989	0.987	0.974	0.965	0.975	0.973	0.977	0.975	0.968	**0.964**	0.976
c_{16}	0.011	0.014	0.009	0.026	0.019	0.013	0.009	0.012	0.012	0.008	0.008	0.016	0.010	0.024	0.007	****	0.993	0.982	0.988	0.979	0.984	0.983	0.983	0.985	0.980	0.973	0.984
c_{17}	0.013	0.017	0.010	0.022	0.018	0.013	0.009	0.010	0.016	0.008	0.011	0.014	0.006	0.016	0.010	0.006	****	0.989	0.977	0.979	0.985	0.982	0.980	0.978	0.982	0.969	0.980
i_1	0.015	0.018	0.023	0.018	0.018	0.022	0.013	0.017	0.014	0.011	0.009	0.020	0.012	0.006	0.012	0.018	0.011	****	0.977	0.981	0.983	0.982	0.980	0.976	0.981	0.974	0.981
i_2	0.010	0.013	0.013	0.023	0.020	0.020	0.007	0.015	0.012	0.017	0.009	0.018	0.022	0.023	0.025	0.012	0.023	0.022	****	0.986	0.988	0.991	0.989	0.991	0.989	0.985	0.991
i_3	0.012	0.018	0.012	0.015	0.014	0.014	0.010	0.007	0.012	0.014	0.011	0.011	0.016	0.021	0.034	0.021	0.020	0.018	0.013	****	0.989	0.994	0.991	0.992	0.992	0.991	0.993
i_4	0.008	0.013	0.014	0.015	0.010	0.013	0.008	0.010	0.007	0.007	0.010	0.008	0.009	0.016	0.024	0.015	0.014	0.017	0.011	0.011	****	0.994	0.990	0.988	0.991	0.988	0.994
i_5	0.004	0.007	0.011	0.012	0.008	0.011	0.007	0.005	0.007	0.008	0.006	0.006	0.010	0.017	0.026	0.016	0.017	0.017	0.008	0.005	0.005	****	0.995	0.997	0.997	0.993	0.996
a_1	0.004	0.008	0.020	0.007	0.008	0.013	0.005	0.006	0.006	0.010	0.013	0.010	0.011	0.013	0.023	0.017	0.020	0.020	0.010	0.008	0.009	0.004	****	0.996	0.991	0.996	**0.998**
a_2	0.005	0.007	0.013	0.014	0.010	0.011	0.009	0.006	0.007	0.010	0.008	0.009	0.013	0.023	0.025	0.014	0.021	0.023	0.008	0.007	0.011	0.002	0.003	****	0.992	0.994	0.996
a_3	0.009	0.013	0.010	0.018	0.014	0.017	0.008	0.005	0.016	0.012	0.009	0.006	0.013	0.016	0.032	0.019	0.017	0.018	0.010	0.007	0.008	0.002	0.008	0.007	****	0.988	0.991
a_4	0.009	0.013	0.027	0.006	0.011	0.015	0.010	0.012	0.007	0.016	0.017	0.014	0.018	0.017	**0.036**	0.027	0.031	0.025	0.014	0.008	0.011	0.006	0.003	0.005	0.011	****	0.996
a_5	0.003	0.008	0.018	0.009	0.008	0.012	0.006	0.008	0.003	0.010	0.010	0.010	0.011	0.016	0.023	0.016	0.020	0.019	0.008	0.006	0.005	0.003	**0.001**	0.003	0.008	0.003	****

4.3. CYTOGENETIC STUDIES

Cytogenetic study was made on the three populations of *Q. aucheri*, two populations of *Q. ilex* and two populations of *Q. coccifera*.

Detailed karyotype analyses of all studied populations were examined. They were observed according to the somatic chromosome number, karyotype formulas, length range, haploid complement, L/S (Largest/Shortest), I^c (Centromeric index), A_1 (Intrachromosomal asymmetry) and A_2 (Interchromosomal asymmetry) values.

Three populations of *Q. aucheri* known as A2, A4 and A5 were karyotyped. The somatic chromosome number of all investigated populations are diploid with 2n=24. Karyotype formulas of all populations are 24 m as well (Figure 25). The averages of chromosomal lengths ranged from 1,05 to 2,06 µm in A2, 0,81 to 1,74 µm in A4 and 1,08 to 2,02 µm in A5 populations (Table 15). Haploid complements of investigated populations are 16,99 µm in A2, 15,05 µm in A4 and 18,15 µm in A5 respectively. Other detailed morphometric parameters like I^c, A_1 and A_2 is shown in Table 16.

When the populations of *Q. aucheri* were compared with each other, results provided from study show the high similarity. All populations have 24 chromosomes and chromosome types are medians. Although A2 and A5 populations show the more similarity than A4 populations according to the length range, haploid complement and L/S values, differences in these parameters are not high level in the comparison to A4. The comparisons of morphometric parameters like I^c, A_1 and A_2 show very similar results too (Table 16). Idiograms of studied *Q. aucheri* populations were presented in Figure 26.

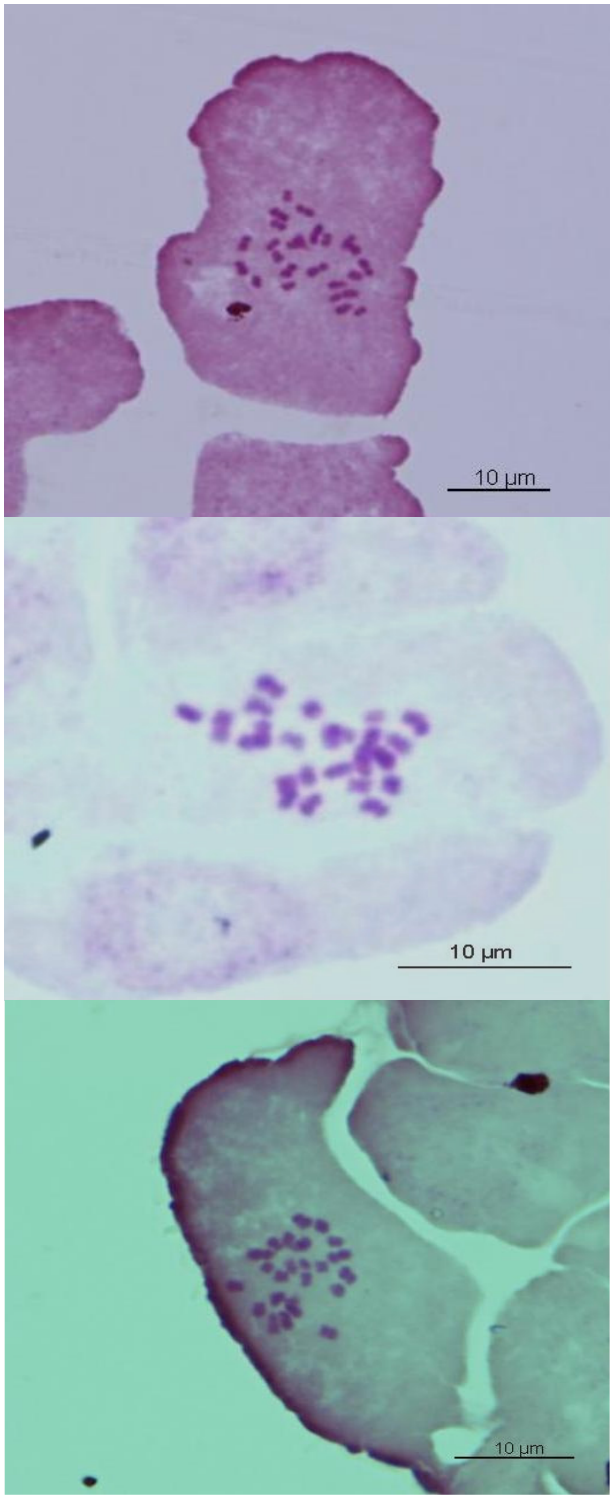

Figure 25- Somatic chromosomes in A2, A4 and A5 populations of *Q. aucheri*, respectively

Table 15- Chromosome numbers, karyotype formulas and length range of the populations of *Q. aucheri*

Quercus aucheri			
Pop. no	**Somatic chromosome number**	**Karyotype formulas**	**Length range(µm)**
a_2	2n=24	24 m.	(1.05–2.06)
a_4	2n=24	24 m.	(0.81–1.74)
a_5	2n=24	24 m.	(1.08–2.02)

Table 16- Morphometric parameters of the populations of *Q. aucheri*. L/S = largest/shortest chromosome; I^c = centromeric index; A_1 = intrachromosomal asymmetry; A_2 = interchromosomal asymmetry. In paranthesis = standart errror

Quercus aucheri					
Pop. no	**Haploid complement (µm)**	**L/S**	**I^c**	**A_1**	**A_2**
a_2	16.99 (±0.08)	1.96 (±0.13)	44.68 (±0,44)	0.19 (±0.01)	0.19 (±0.08)
a_4	15.05 (±0.07)	2.14 (±0.15)	45.34 (±0.36)	0.17 (±0.01)	0.20 (±0.07)
a_5	18.15 (±0.07)	1.87 (±0.11)	45.45 (±0.51)	0.17 (±0.01)	0.17 (±0.07)

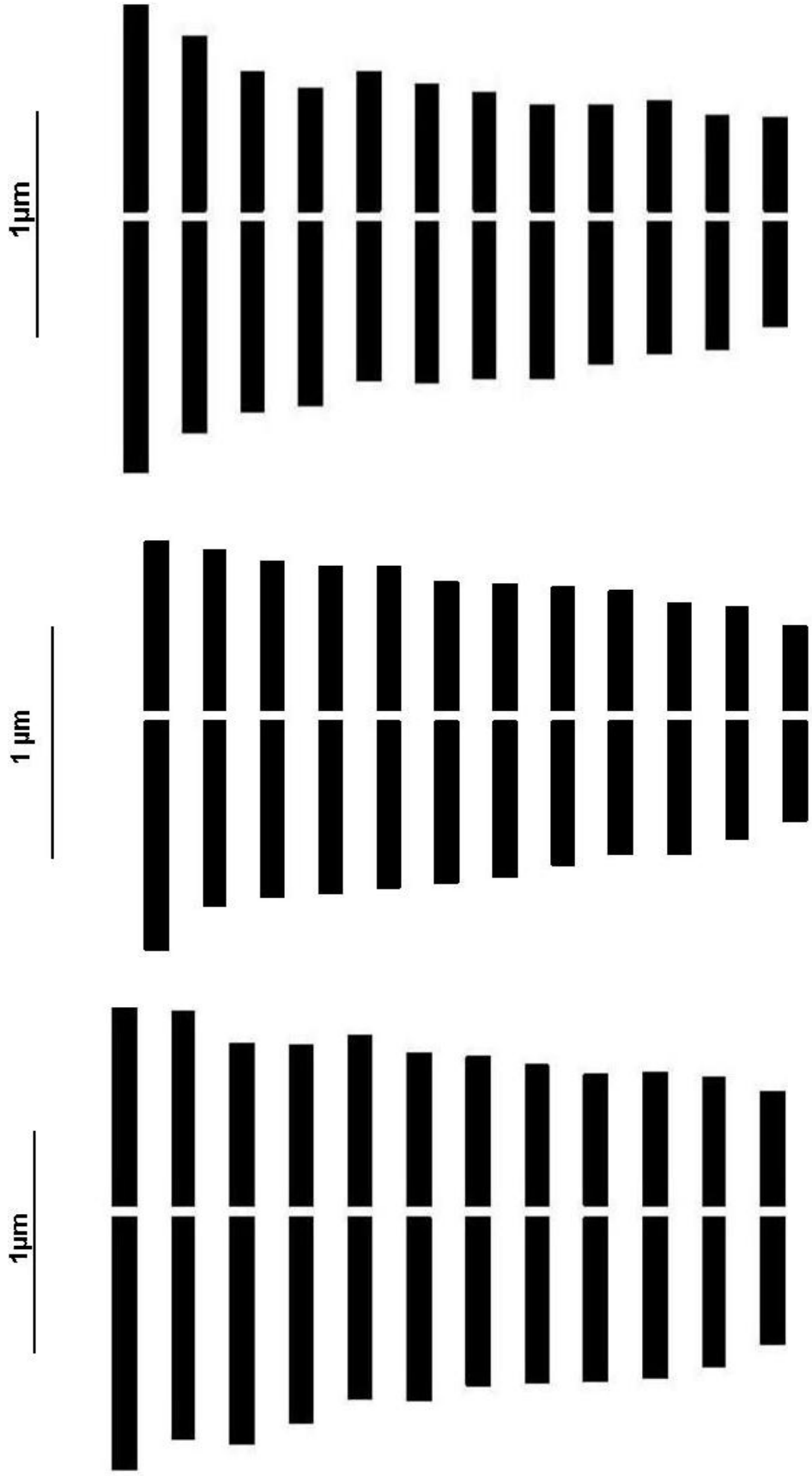

Figure 26- Idiograms of A2, A4 and A5 populations of *Q. aucheri*, respectively

Detailed karyotype analyses of *Q. ilex* were examined on two populations. These are I2 and I3 populations. The somatic chromosome number of these two populations are 2n=24 and karyotype formulas are 24 m presented in Figure 27. The averages of chromosomal lengths ranged from 1,07 to 2,05 µm in I2 and 1,18 to 2,13 µm in I3 populations (Table 17). Haploid complements of I2 and I3 are 17,47 µm and 18,20 µm, respectively. Other morphometric parameters like L/S, I^c, A_1 and A_2 is shown in Table 18.

The populations of *Q. ilex* show the high similarity according to the morphometric parameters. In the comparison to I2 and I3 populations according to the length range, haploid complement, L/S, I^c, A_1 and A_2, it can be clearly said that I2 and I3 are very similar. Idiograms of studied *Q. ilex* populations were presented in Figure 28.

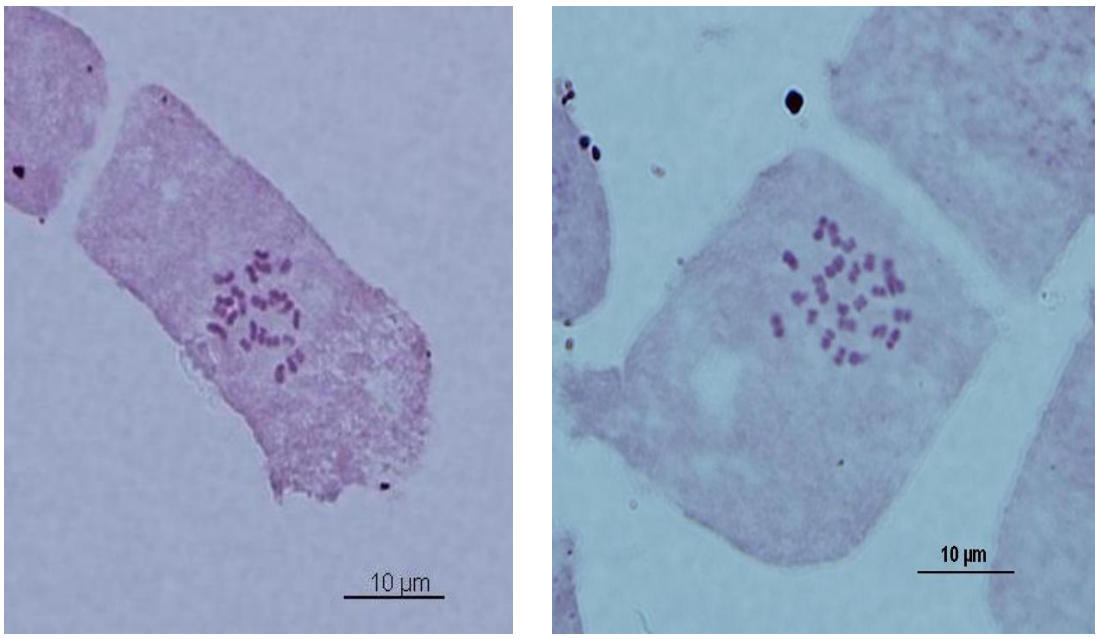

Figure 27- Somatic chromosomes in I2 and I3 populations of *Q. ilex*, respectively

Table 17- Chromosome numbers, karyotype formulas and length range of the populations of *Q. ilex*

Quercus ilex			
Pop. no	**Somatic chromosome number**	**Karyotype formulas**	**Length range(µm)**
i_2	2n=24	24 m.	(1.07–2.05)
i_3	2n=24	24 m.	(1.18–2.13)

Table 18- Morphometric parameters of the populations of *Q. ilex*. L/S = largest/shortest chromosome; I^c = centromeric index; A_1 = intrachromosomal asymmetry; A_2 = interchromosomal asymmetry. In paranthesis = standart errror

Quercus ilex					
Pop. no	**Haploid complement (µm)**	**L/S**	**I^c**	**A_1**	**A_2**
i_2	17.47 (±0.08)	1.91 (±0.12)	44.10 (±0,35)	0.21 (±0.01)	0.20 (±0.08)
i_3	18.20 (±0.07)	1.80 (±0.11)	42.75 (±0.77)	0.23 (±0.02)	0.17 (±0.07)

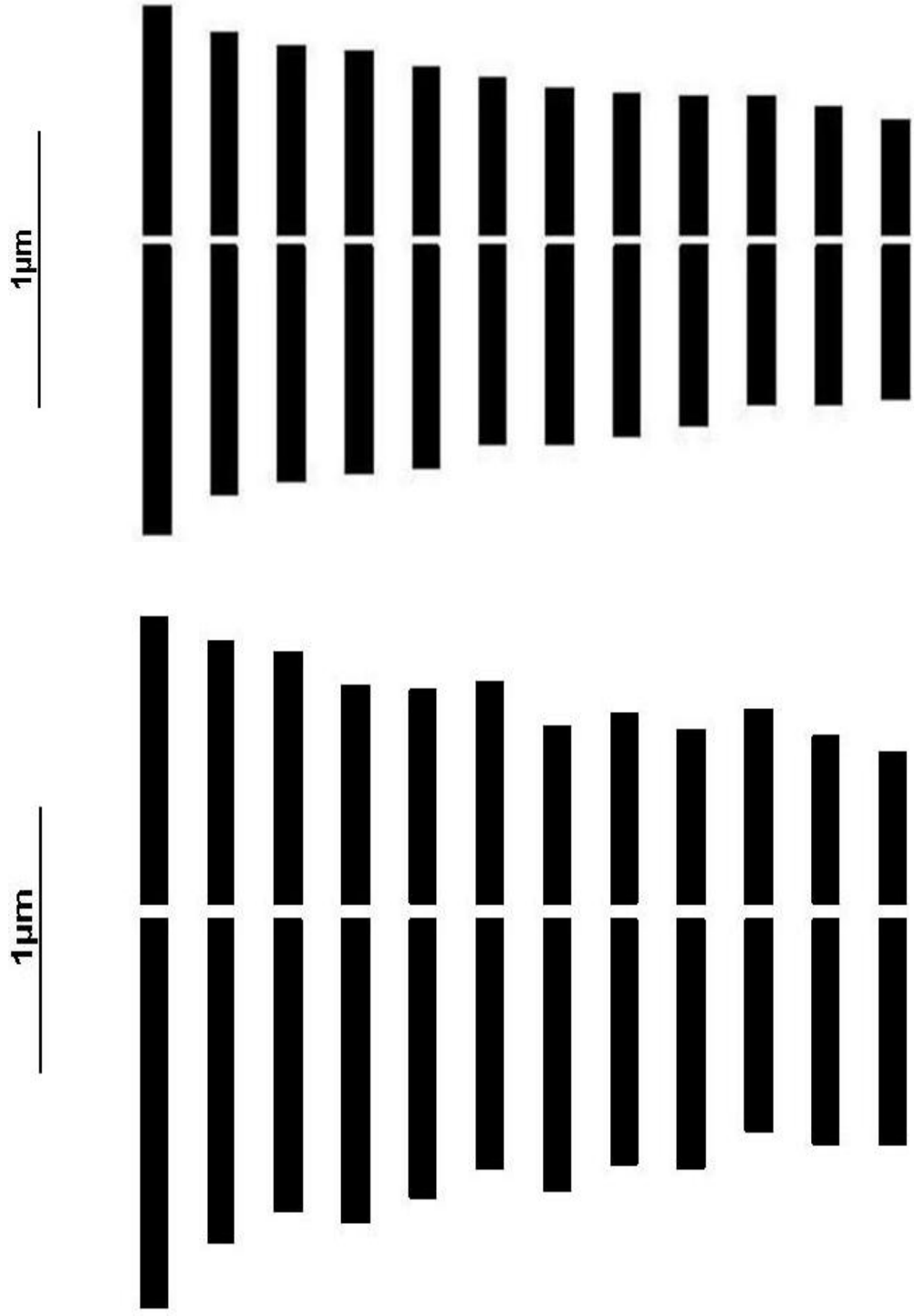

Figure 28- Idiograms of I2 and I3 populations of *Q. ilex*, respectively

Q. coccifera was examined on two populations known as C13 and C17. Somatic chromosome number of these populations are diploid with 2n=24 and karyotypic descriptions are 24 m in Figure 29. The averages of chromosomal lengths ranged from

0,80 µm to 1,98 µm in C13 and 0,81 µm to 2,13 µm in C17 populations (Table 19). Haploid complements of the investigated populations are 14,61 µm in C13 and 15,30 µm in C17. Other morphometric parameters show the high similarity in C13 and C17 populations of *Q. coccifera* (Table 20).

When the C13 and C17 populations are compared with each other, it is observed the high similarity in all morphometric parameters. Idiograms of studied *Q. coccifera* populations were presented in Figure 30.

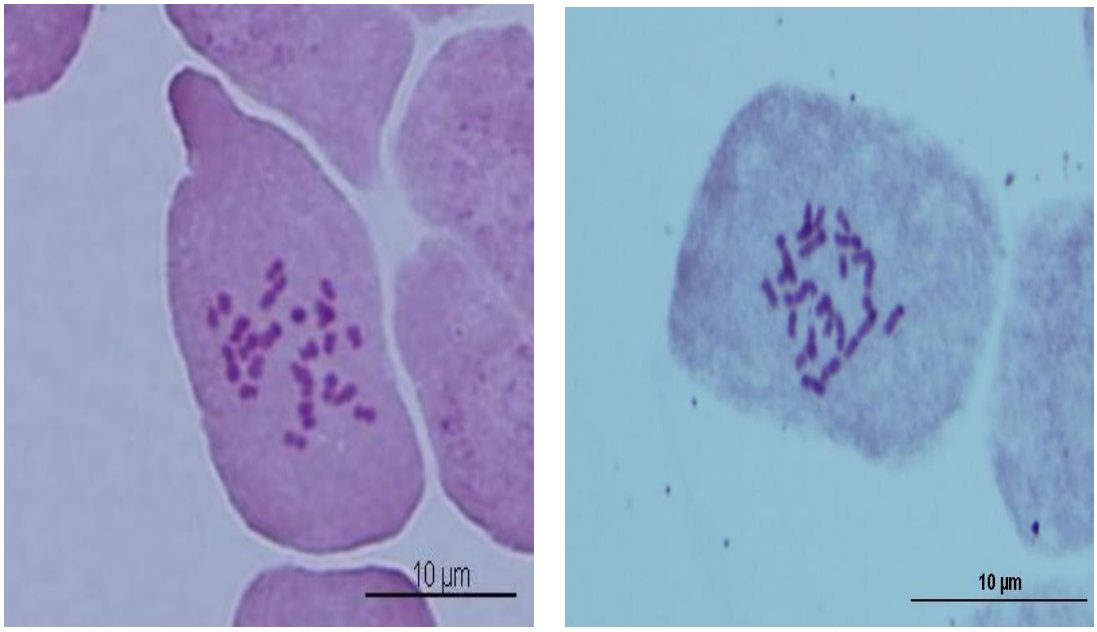

Figure 29- Somatic chromosomes in C13 and C17 populations of *Q. coccifera*, respectively

Table 19- Chromosome numbers, karyotype formulas and length range of the populations of *Q. coccifera*

Quercus coccifera			
Pop. no	**Somatic chromosome number**	**Karyotype formulas**	**Length range(µm)**
c_{13}	2n=24	24 m.	(0.80–1.98)
c_{17}	2n=24	24 m.	(0.81–2.13)

Table 20- Morphometric parameters of the populations of *Q. coccifera*. L/S = largest/shortest chromosome; I^c = centromeric index; A_1 = intrachromosomal asymmetry; A_2 = interchromosomal asymmetry. In paranthesis = standart errror

Quercus coccifera					
Pop. no	**Haploid complement (µm)**	**L/S**	**I^c**	**A_1**	**A_2**
c_{13}	14.61 (±0.09)	2.47 (±0.21)	44.80 (±0,37)	0.19 (±0.01)	0.27 (±0.10)
c_{17}	15.30 (±0.10)	2.62 (±0.23)	44.18 (±0,50)	0.21 (±0.01)	0.29 (±0.10)

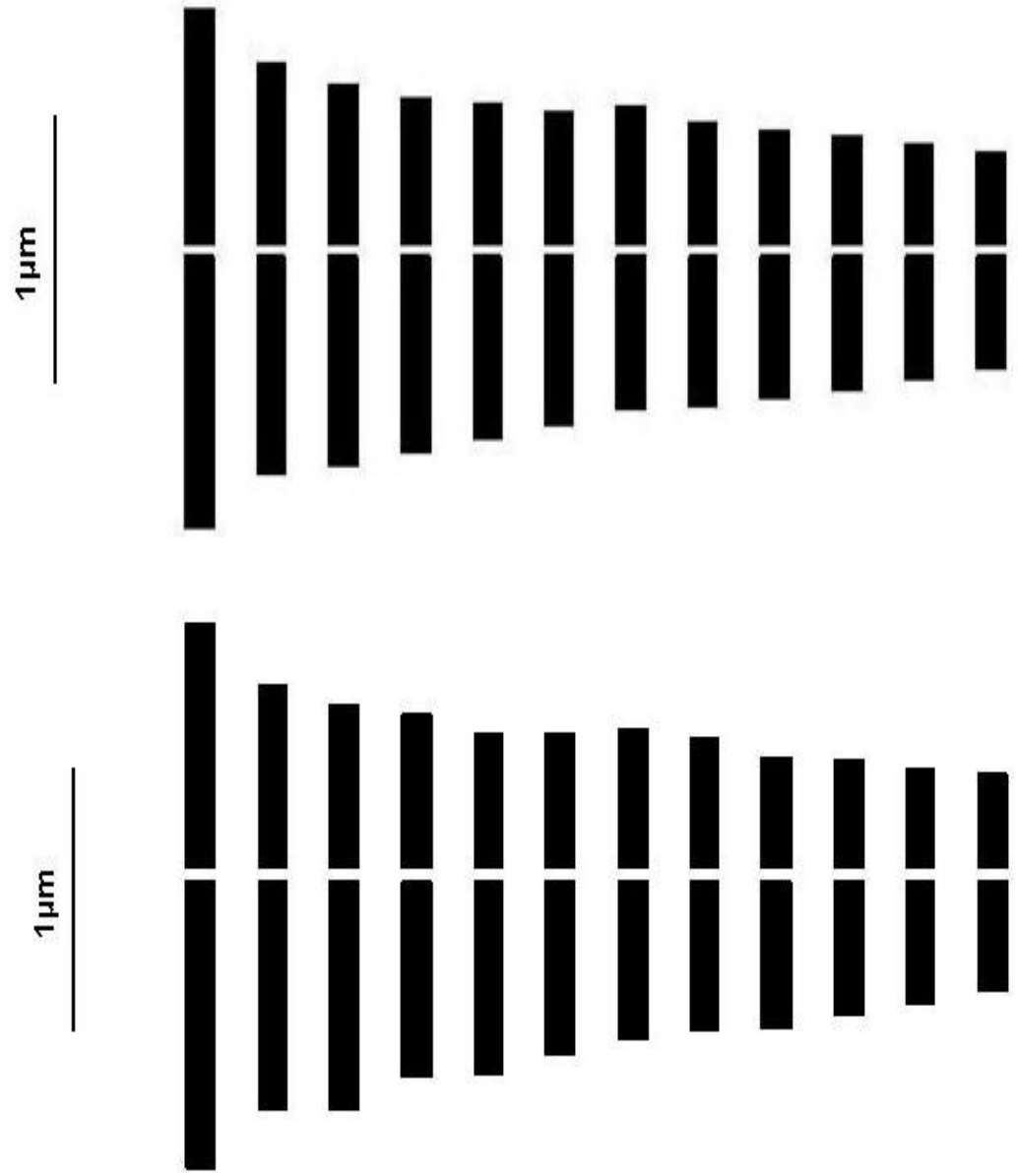

Figure 30- Idiograms of C13 and C17 populations of *Q. coccifera*, respectively

CHAPTER V

5. DISCUSSION

Disscusions about the results of morphometric, RAPD-PCR, and cytogenetics studies are carried out separately.

5.1. DISCUSSION OF MORPHOLOGICAL STUDIES

Results provided from fruit characters in CA analyses are showed that three taxa belonging to *Ilex* section separated same as leaf results (Figure 11). According to CA results of fruit characters (Figure 11) *Q. ilex* shows the more differentiation than other species. It is observed the presence of the second group within *Q. coccifera*. These are populations located on East Mediterranean Region.

When PCA results are evaluated, these did not show clear separation like CA (Figure 12). But when Figure 13 is observed, result is very compatible with CA. In Figure 13, the seperation of three taxa belonging to *Ilex* section is observed more clearly than Figure 12. Leaf materials are often used for the morphometric measurement studies in oaks (Borazan and Babaç, 2003; Bruschi et al., 2000; Kremer et al., 2002; Boratynski et al., 2008; Gonzalez-Rodriguez and Oyama, 2005; Bruschi et al., 2003; Gonzalez-Rodriguez et al., 2004; Franjic et al., 2006; Ponton et al., 2004). But we did not see many studies using fruits for morphometric measurements in oaks. In this study CA and PCA analysis were used together for fruit and leaf samples and the results are found to

be very compatible with each other.

Among the *Q. ilex* populations with leaf characters, the most difference is observed in I5 population (Figure 9). The locality of this population is an island in Aegean sea. In other words, I5 is isolated population and as a result of this it showed differences from the rest populations. Second main group consists of populations belonging to *Q. coccifera* and *Q. aucheri* in Figure 9. The highest variation among these populations was observed in C7 population according to CA results. This difference may be caused by high altitude of C7 population locality. When the localities of all populations are examined, C7 population has the second highest altitude. Populations of *Q. aucheri* show the differences from populations of *Q. coccifera* within the second main group. But this difference is not clear like *Q. ilex* (Figure 9-10). The C5 population is located in Aegean sea as an island. This could be the main reason for being different from the rest of *Q. coccifera* populations. Other population C17 is located in Hatay which is placed in the East Mediterranean region.

As it has been previously stated; *Q. coccifera* is confused with *Q. calliprinos*. Mostly these two species show the differences in geographical distribution. According to the Toumi (1995), while *Q. coccifera* is located on West Mediterranean region, *Q. calliprinos* on East Mediterranean region and these two species may be evaluated within *Q. cocifera* as *Q. coccifera* subsp. *coccifera* and *Q. coccifera* subsp. *calliprinos*. Situation of C17, C16, C15 and C14 populations are supporting the second sub-gruop among the populations of *Q. coccifera*. Population of C14 (Yalova) is geographically located on the west part of the country, but it was found among the East Mediterranean region sub-group of *Q. coccifera*. There might be different reasons for this result. One of them is, Yalova population (C14) could be an artificial, as trees were in a protected

area.

Finally, species are separated from each other and especially within *Q. coccifera* the presence of the second group is observed in both leaf and fruit character analysis. It was observed that the populations showing the differences within *Q. coccifera* are located on East Mediterranean Region.

5.2. DISCUSSION OF MOLECULAR STUDIES

Genetic diversity of all studied populations was found Ht= 0,383 and genetic differentiation was Gst= 0,020 in this study. In a study of between *Q. robur* and *Q. petraea*, this value Ht= 0,223 was found (Kelleher et al., 2005). In the same research the species population diversity was also found different such as *Q. robur* Ht= 0,264 and *Q. petraea* Ht= 0,243 (Kelleher et al., 2005). The same difference among the species diversity was also observed in our study but in less extent *Q. aucheri* Ht= 0,382, *Q. ilex* Ht= 0,379 and *Q. coccifera* Ht=0.371.

In some other research Gst was found between 0,07-0,27 in *Q. pubescens* populations of Croation (Franjic et al., 2006). Our Gst result was found less than these values. According to these results differentiation of *Ilex* section taxa has less than those *Quercus* section taxa.

Gene flow value (Nm) was found very high 23,55 in our study. Some other researchers found these values between 0,70-14,37 in Croation populations (Franjic et al., 2006). This could be the result of high outbreeding occurred among *Ilex* section taxa.

According to AMOVA results, variations among the populations 71,59 % and

within the populations 28,41% was found. In some *Q. petraea* populations these results were 20% and 80% among and within the populations (Bruschi et al 2003). In another study between the *Q. robur* and *Q. petraea* AMOVA was found 38% among the populations and 69% within the populations (Kelleher et al., 2005). In our study variations was found among the populations higher than those within populations. One of the reason for that, it could be the results of three different taxa involved in the study.

The highest genetic similarity is observed between C1-C2 and A1-A5 populations. The localities of these populations are very close to each other. Both populations located on the same region (İzmir). Similarly, A1 and A5 populations are very close two populations like C1 and C2 (Table 14). On the other hand genetically distant two pair populations (C15-A4 and C15-A3) are belong to different taxa. Furthermore, these genetically distant populations are also located geographically in different regions.

This is the first report of RAPD data analysis for assessing relationships between these three taxa. But there are some studies used RAPD data in different sections of oaks (Franjic et al., 2006; Bruschi et al., 2003).

The molecular analysis with CA and PCA revealed a high degree of separation between the species. Here on the contrary of morphological study, *Q. ilex* and *Q. aucheri* showed more similarity. *Q. coccifera* was separated into three groups. It was observed that the populations close to each other showed high similarity on the contrary of distant populations. According to the CA and PCA produced from RAPD results , it clearly observed that populations having the highest differences within *Q. coccifera* are C14, C15, C16 and C17 (Figure 23-24). This finding is similar with morphometric

results of leaf and fruits. But when the results of morphological and molecular study for CA and PCA analysis are evaluated together, the results of molecular study are more meaningful than those of morphological study.

5.3. DISCUSSION OF CYTOGENETIC STUDIES

The chromosome numbers, karyotype formulas and morphometric parameters of all studied populations were determined. Purpose of this part of the study to see whether there is any chromosomal differences in different populations of the same taxon. Nevertheless karyotypic analyses of these populations were found to be very similar having all median type chromosomes. The somatic chromosome numbers of all investigated populations are diploid with 2n=24 (Figures 25, 27 and 29).

When the different taxon results compared with the parameters of haploid complement, it was found that C13 population of *Q. coccifera* has the smallest chromosomes in Table 20, I3 population of *Q. ilex* has the biggest chromosomes in Table 18. In other words; the populations of *Q. coccifera* have the smallest value according to parameter of haploid complement. However, the populations of *Q. ilex* have the highest value.

Centromeric index (I^c) is very close in all populations of three species (Table 16, 18 and 20). In the same way, *Q. aucheri*, *Q. ilex* and *Q. coccifera* showed very similar intrachromosomal asymmetry (A_1). On the contrary to intrachromosomal asymmetry, interchromosomal asymmetry (A_2) was found to be a little distinct in *Q. coccifera*. But fundamentally, all populations of the studied taxa showed very similar interchromosomal asymmetry.

When the results obtained from this study are compared with previous studies on

the chromosome numbers of *Quercus* taxa do not show much variations except *Q. petraea*. Chromosome number of *Q. petraea* may be 2n=24+1, 2, 3 (Zoldo et al., 1998). Previous reports related to karyotype on *Quercus* show that all species of *Quercus* investigated have diploid chromosome with 2n=24 (Zoldo et al., 1998; Duffield, 1940; Stairs, 1964; Kurokawa and Yonezawa, 2004; Demerico et al., 1995-2000; Ohri and Ahuja, 1990). Our studied *Quercus* chromosome number findings verify 2n=24.

When results provided from our study are compared with other studied species from the point of morphometric parameters, they are not entirely mathced (Demerico et al., 1995). Nevertheless, they are not completely very distant from those values either. But when studied species are evaluated with each other in this study, morphometric parameters show less distinction. In general, our results showed very least parametric values from *Quercus* species studied by Demerico et al. (1995-2000).

In this study, karyotypes of taxa belonging to *Ilex* section have been done for the first time. All species studied have diploid chromosome (2n=24) with similar morphometric parameters.

CHAPTER VI

6. CONCLUSION

In Turkey, section *Ilex* is composed of three species, *Q. coccifera*, *Q. aucheri* and *Q. ilex* (Davis, 1982). Taxonomy of this group is not studied well. Especially *Q. aucheri* is known as "forgotten oak tree'' (Yaltırık, 1984), because it is distributed only in South West Turkey and in a few East Aegean Islands (Davis, 1982). By this study; lacking of the morphology, molecular and cytogenetic properties of *Ilex* section are completed in detail.

The results of morphometric, molecular and cytogenetic studies gave the satisfactory findings for phenetic groupings of taxa in *Ilex* section. The most significant differences are found on the *Quercus ilex* populations. This taxon is separated from the remaining species in both morphological and molecular results. According to the results of morphometric analyses, due to similarities among the some *Q. coccifera* and *Q. aucheri* populations, did not clearly separated from each other. However, they are well separated with the results of molecular study. In addition, *Quercus coccifera* populations are grouped next to *Q. aucheri* populations in Cluster Analyses (CA) (Figure 9-11 and 23).

It is the first time presented in this study that *Q. coccifera* is separated into two geographical groups in Turkey. The first group has the populations sampled from Southern Marmara, Northern Aegean, Southern Aegean and Western Mediterranean

regions. The populations sampled from C14, C15, C16 and C17 Eastern Mediterranean region included into the second group. Among the second group populations, C14 (Yalova) is located in the South Marmara region of the country, but it was grouped among the Eastern Mediterranen region. Both morphological and molecular results supported these groupings. This could be the result of an unnatural population as C14 was located in the protected private area. This geographically separation of *Q. coccifera* suggests that there are at least sub-groupings or different species in this taxon. The most common group is *Q. coccifera* found in many regions, while the less and restricted group is found only in the East Mediterranean region. The second group is geographically closer to Syria, Israel and Palestine. These groupings were also suggested by Toumi (1995). In addition, these two groups are represented as a single *Q. calliprinos* species and its two subspecies as *Q. calliprinos* subsp. *coccifera* and *Q. calliprinos* subsp. *calliprinos* in Flora of Palestine (Zohary, 1966).

In the molecular part of the study, in addition to CA and Principle Component Analysis (PCA), other analyses were made showing differences within and among populations like genetic diversity (Ht), genetic differentiation (Gst), gene flow (Nm) and genetic similarity. In our study, generally Ht and Gst values are less than those of European oaks (Table 13) (Kelleher et al., 2005; Franjic et al., 2006). Genetic diversity is Ht= 0,383 and genetic differentiation is Gst= 0,020. But gene flow is found very high Nm= 23,552 among the studied populations. Gene flow is an important feature of population genetics, shaping the diversity of species (Dumolin-Lepague et al, 1999; Jensen et al, 1993). Out-breeding might occurred more often among the species of *Ilex* section than those of *Quercus* section. This could be the reason that gene flow is found very high among the *Ilex* section species in this study. When the studied populations are

compared with each other according to the genetic similarity, it can be said that genetically distant populations are also located geographically in different and far regions (Table 14). The most high genetic similarity are found between C1-C2 and A1-A5 populations which are also geographically close populations. On the contrary, genetically the most distant populations, C15-A4 and C15-A3 are the two different species which are geographically located very distant. These results are also supported by the morphometric analyses of leaf and fruit characters.

According to the result of cytogenetic study, populations of three species were found to be very similar. This study provided that the somatic chromosome numbers of investigated populations are diploid with 2n=24. *Quercus coccifera* chromosome number has already been reported from Turkey (Yılmaz et al 2008). The studied populations have consisted of median type chromosomes. According to the centromeric index (I^c) and intrachromosomal asymmetry (A_1); *Q. aucheri*, *Q. ilex* and *Q. coccifera* showed very similar results (Table 16-18 and 20). However, interchromosomal asymmetry (A_2) was showed that a minute alteration in *Q. coccifera*. Besides this finding, *Q. coccifera* had the smallest value of haploid complement (Table 20).

As a result of this study, it might be suggested that,

1. The results showed the presence of the second group within *Q. coccifera* but this needs to be supported in a study including *Q. calliprinos* samples from Syria, Israel and Palestina.

2. The groupings based on morphological and molecular studies support the presence of the three species in *Ilex* section. However, *Q. coccifera* is separated from the remaining taxa in cytogenetic analyses.

3. The two groups showing geographical differentiations within *Q. coccifera* may strengthen of existence two infraspecific taxa such as *Q. coccifera* subsp. *coccifera* and subsp. *calliprinos* or two different taxa at species level.

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APPENDICES

APPENDIX 1 - CODIFYING METHODS FOR THE FRUIT CHARACTERS

THE MEANS OF ALL POPULATIONS FOR EACH CHARACTERS IN FRUITS

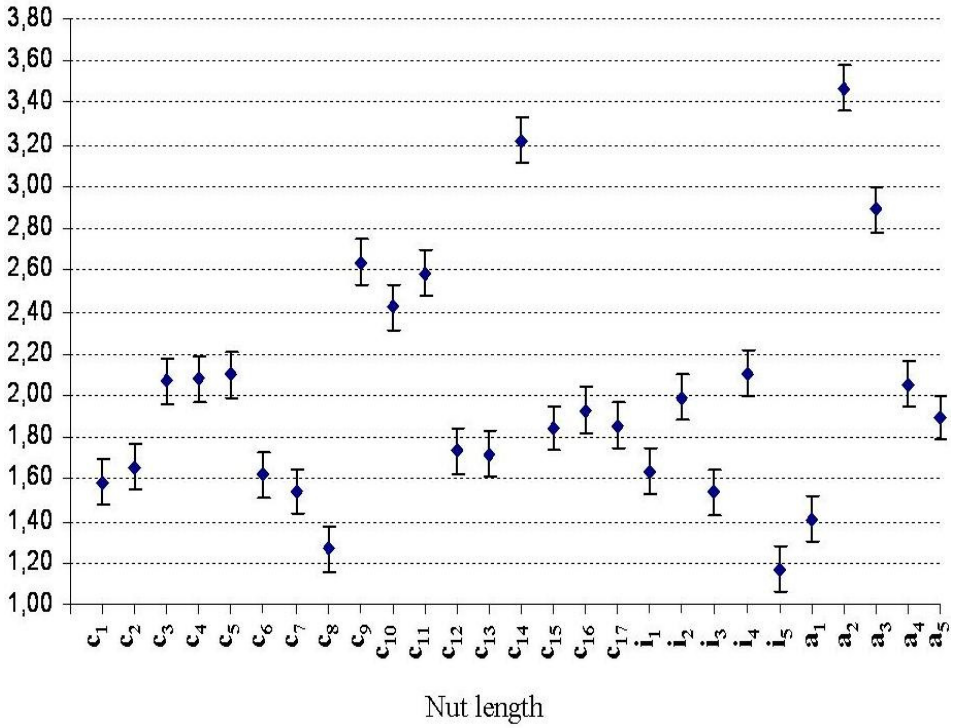

Figure 31- Means with standart error plots for nut length

Table 21- Scoring character states for nut length

Group number	Coding range	Character state
1	0– 2,20	1
2	2,20– +∞	2

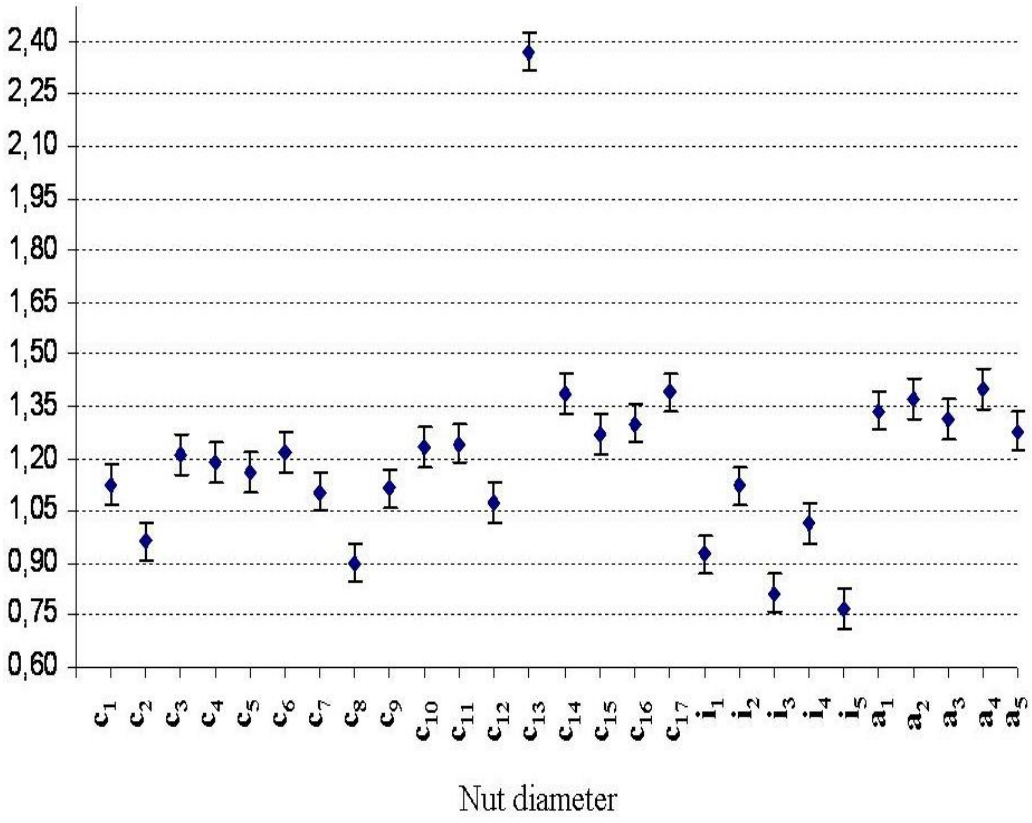

Figure 32- Means with standart error plots for nut diameter

Table 22- Scoring character states for nut diameter

Group number	Coding range	Character state
1	0–1,25	1
2	1,25–1,65	2
3	1,65– +∞	3

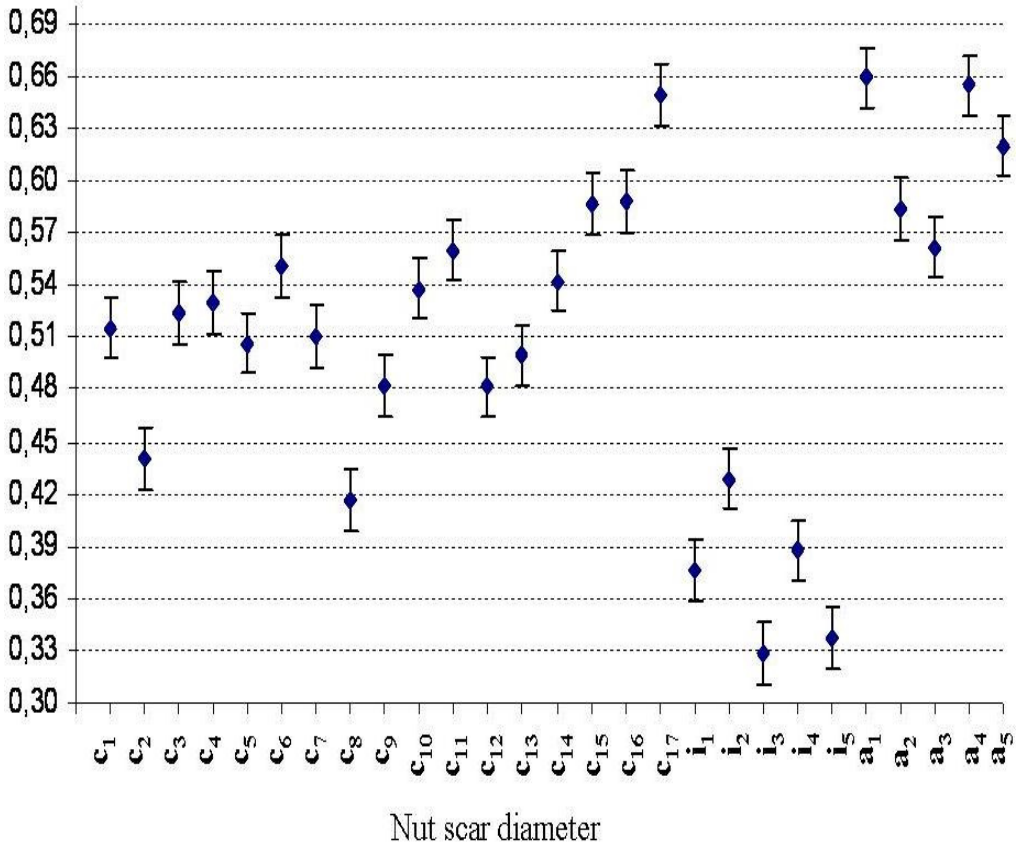

Figure 33- Means with standart error plots for nut scar diameter

Table 23- Scoring character states for nut scar diameter

Group number	Coding range	Character state
1	0–0,43	1
2	0,43–0,55	2
3	0,55– +∞	3

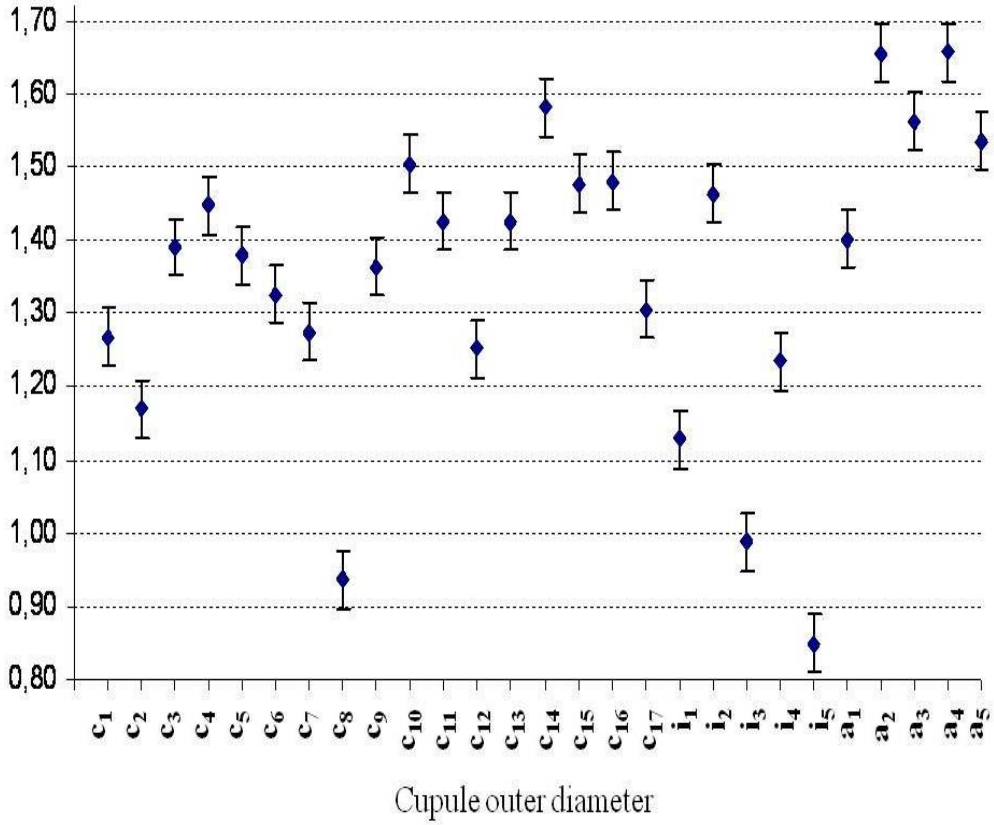

Figure 34- Means with standart error plots for cupule outer diameter

Table 24- Scoring character states for cupule outer diameter

Group number	Coding range	Character state
1	0–1,24	1
2	1,24–1,45	2
3	1,45– +∞	3

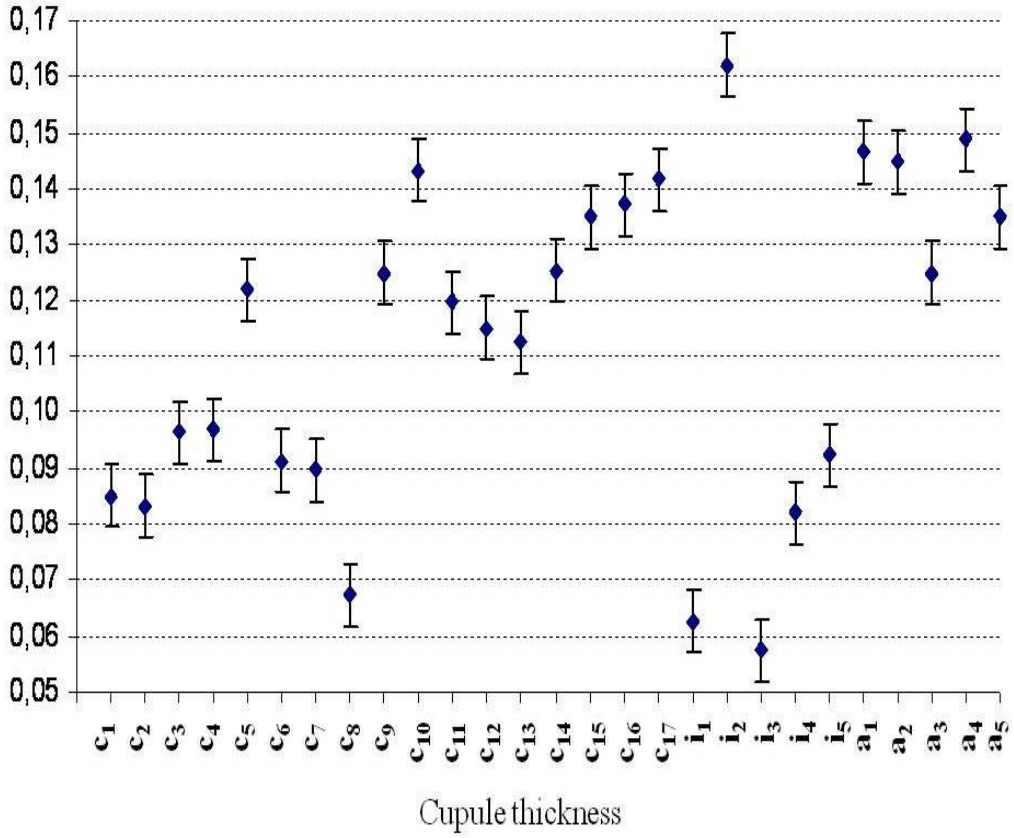

Figure 35- Means with standart error plots for cupule thickness

Table 25- Scoring character states for cupule thickness

Group number	Coding range	Character state
1	0–0,10	1
2	0,10–0,15	2
3	0,15– +∞	3

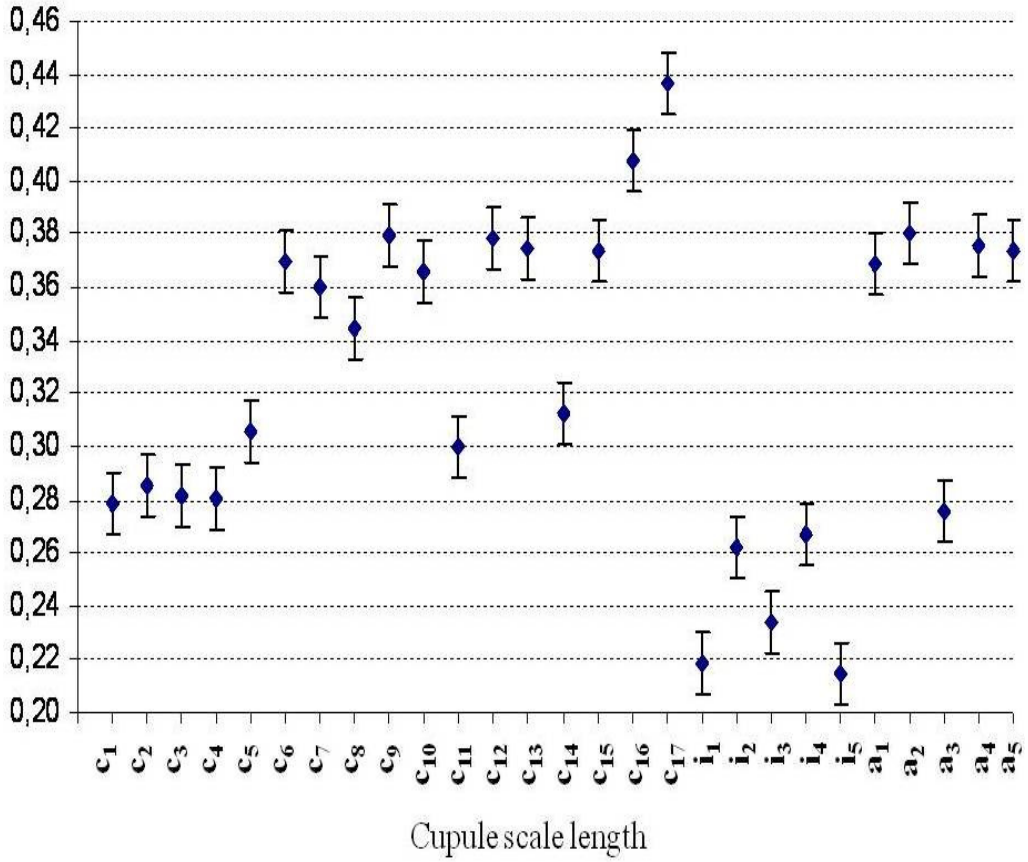

Figure 36- Means with standart error plots for cupule scale length

Table 26- Scoring character states for cupule scale length

Group number	Coding range	Character state
1	0–0,27	1
2	0,27–0,32	2
3	0,32–0,40	3
4	0,40– +∞	4

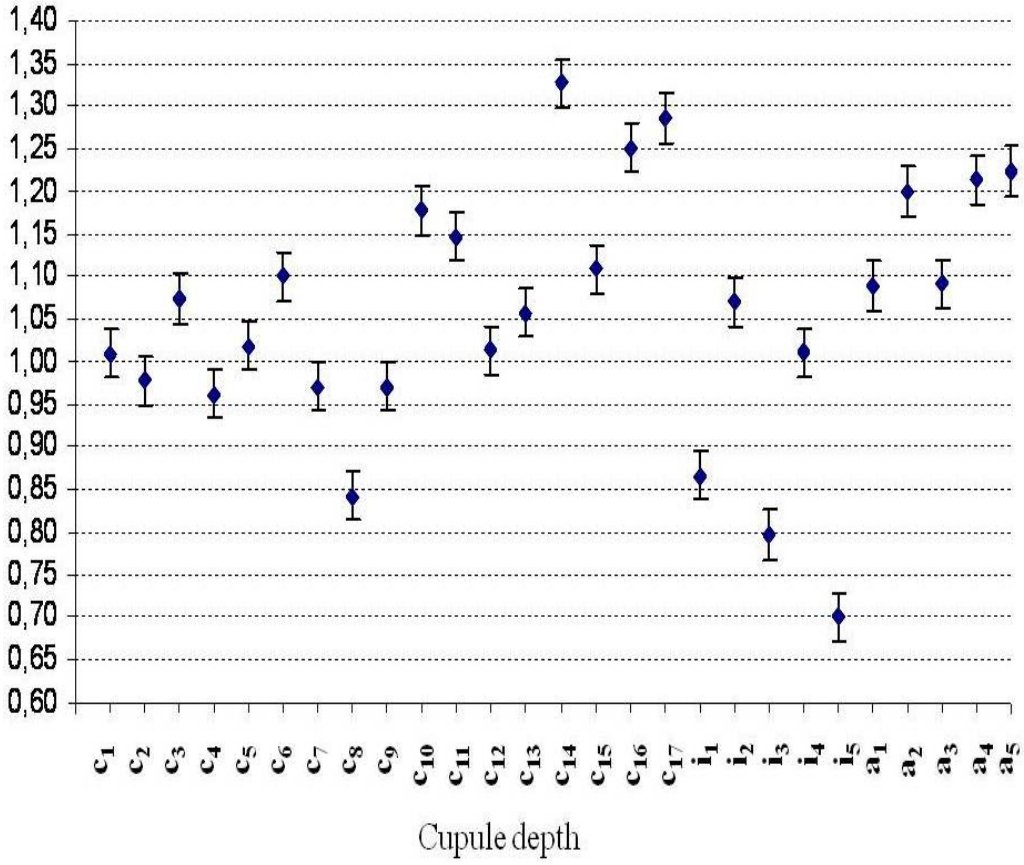

Figure 37- Means with standart error plots for cupule depth

Table 27- Scoring character states for cupule depth

Group number	Coding range	Character state
1	0–1,08	1
2	1,08–1,23	2
3	1,23– +∞	3

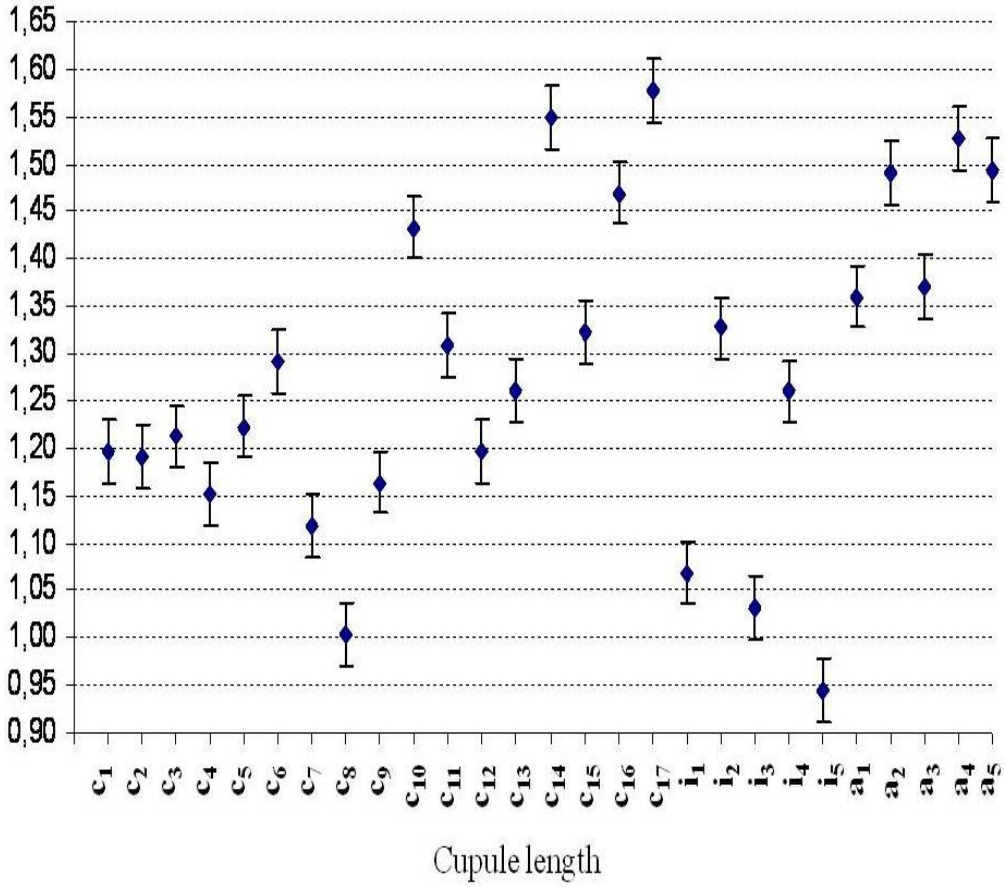

Figure 38- Means with standart error plots for cupule length

Table 28- Scoring character states for cupule length

Group number	Coding range	Character state
1	0–1,34	1
2	1,34– +∞	2

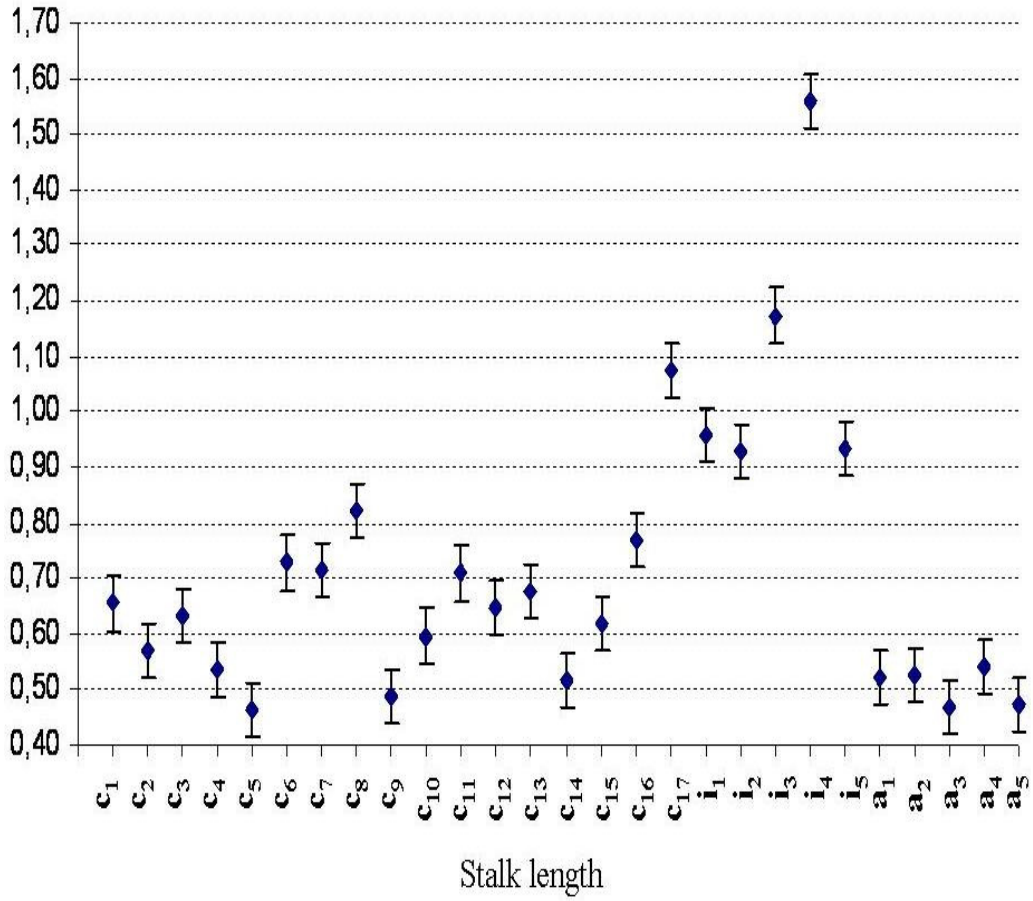

Figure 39- Means with standart error plots for stalk length

Table 29- Scoring character states for stalk length

Group number	Coding range	Character state
1	0–0,85	1
2	0,85–1,30	2
3	1,30– +∞	3

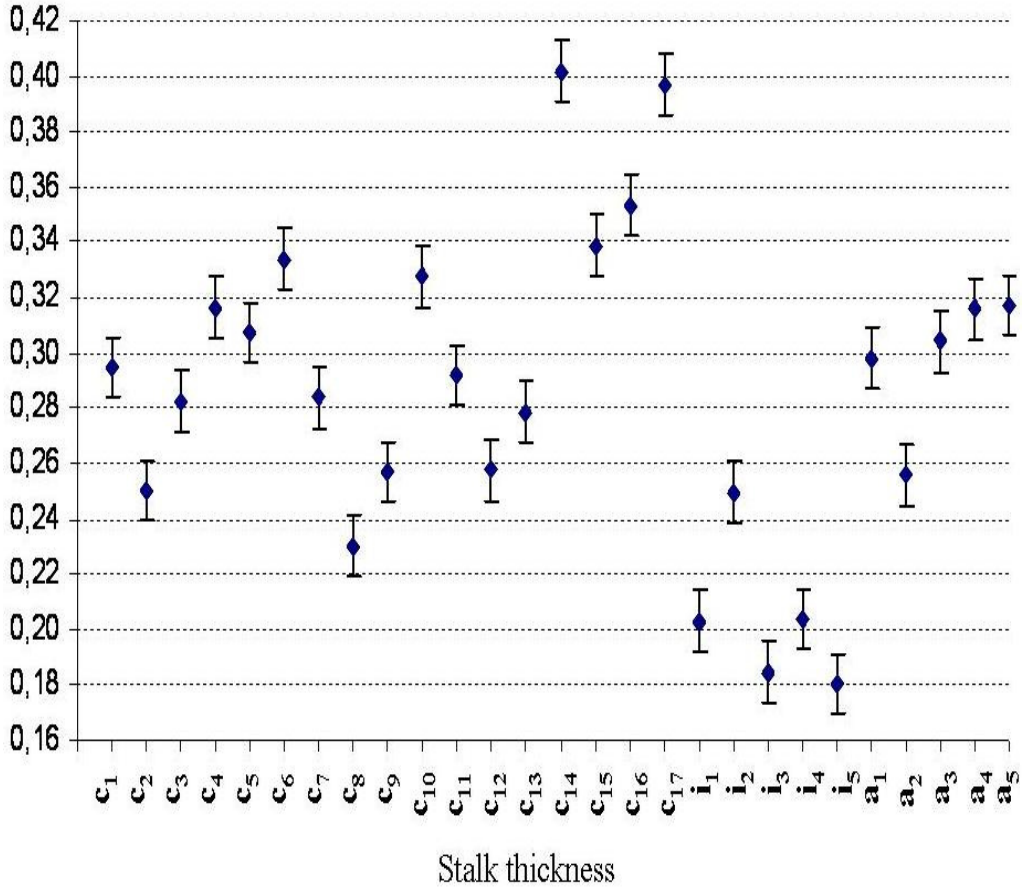

Figure 40- Means with standart error plots for stalk thickness

Table 30- Scoring character states for stalk thickness

Group number	Coding range	Character state
1	0–0,25	1
2	0,25–0,33	2
3	0,33– +∞	3

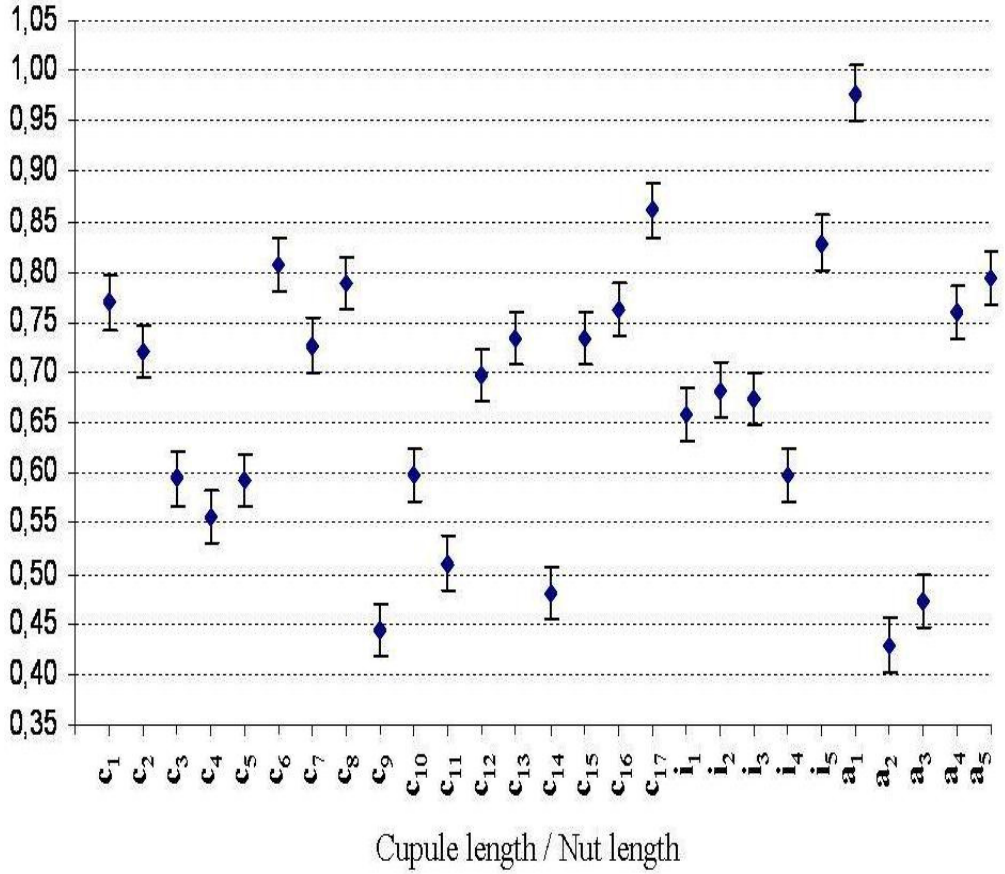

Figure 41- Means with standart error plots for cupule length/nut length

Table 31- Scoring character states for cupule length/nut length

Group number	Coding range	Character state
1	0–0,57	1
2	0,57–0,82	2
3	0,82– +∞	3

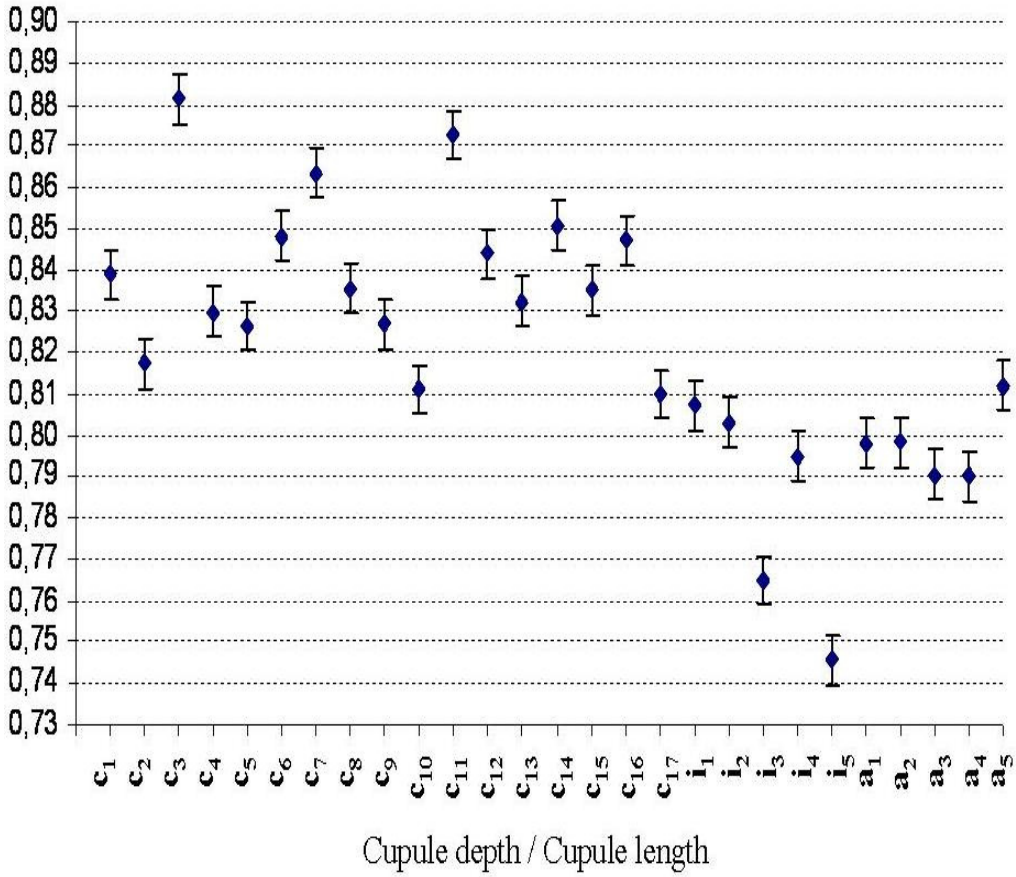

Figure 42- Means with standart error plots for cupule depth/cupule length

Table 32- Scoring character states for cupule depth/cupule length

Group number	Coding range	Character state
1	0–0,78	1
2	0,78–0,82	2
3	0,82–0,86	3
4	0,86– +∞	4

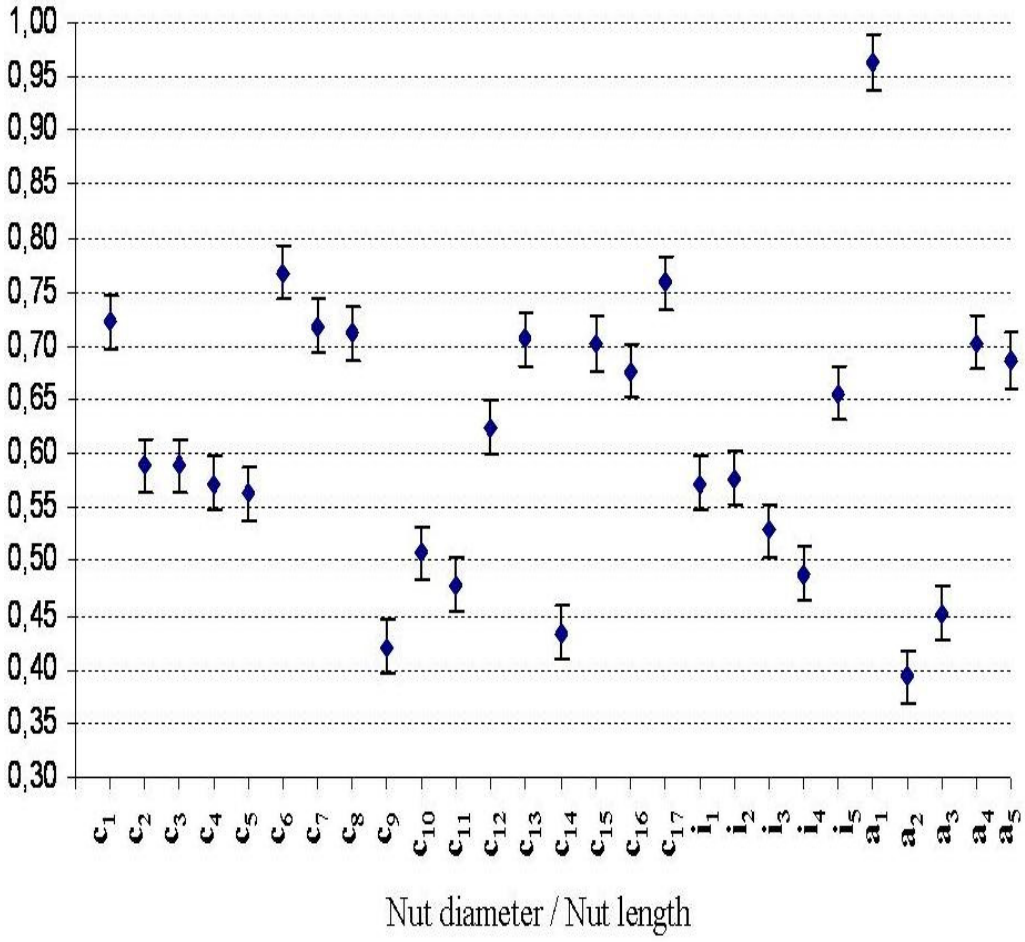

Figure 43- Means with standart error plots for nut diameter/nut length

Table 33- Scoring character states for nut diameter/nut length

Group number	Coding range	Character state
1	0–0,61	1
2	0,61–0,80	2
3	0,80– +∞	3

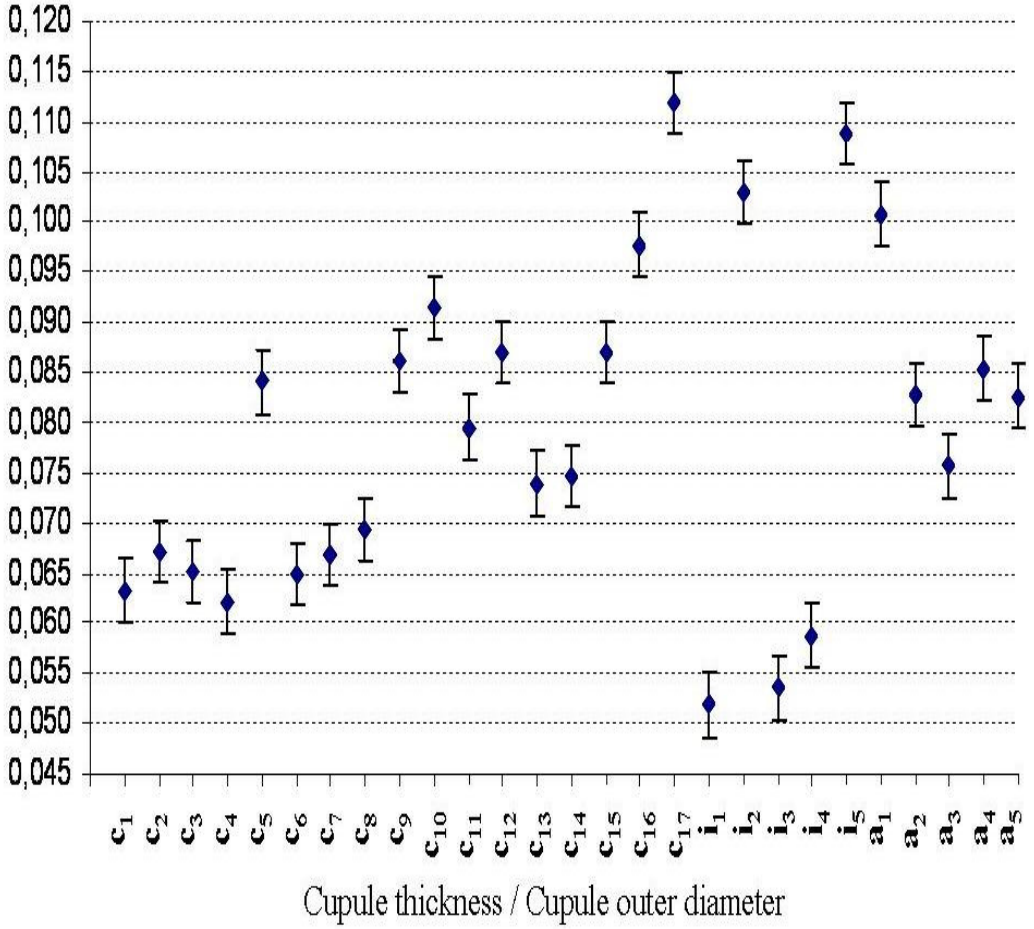

Figure 44- Means with standart error plots for cupule thickness/cupule outer diameter

Table 34- Scoring character states for cupule thickness/cupule outer diameter

Group number	Coding range	Character state
1	0–0,072	1
2	0,072–0,095	2
3	0,095– +∞	3

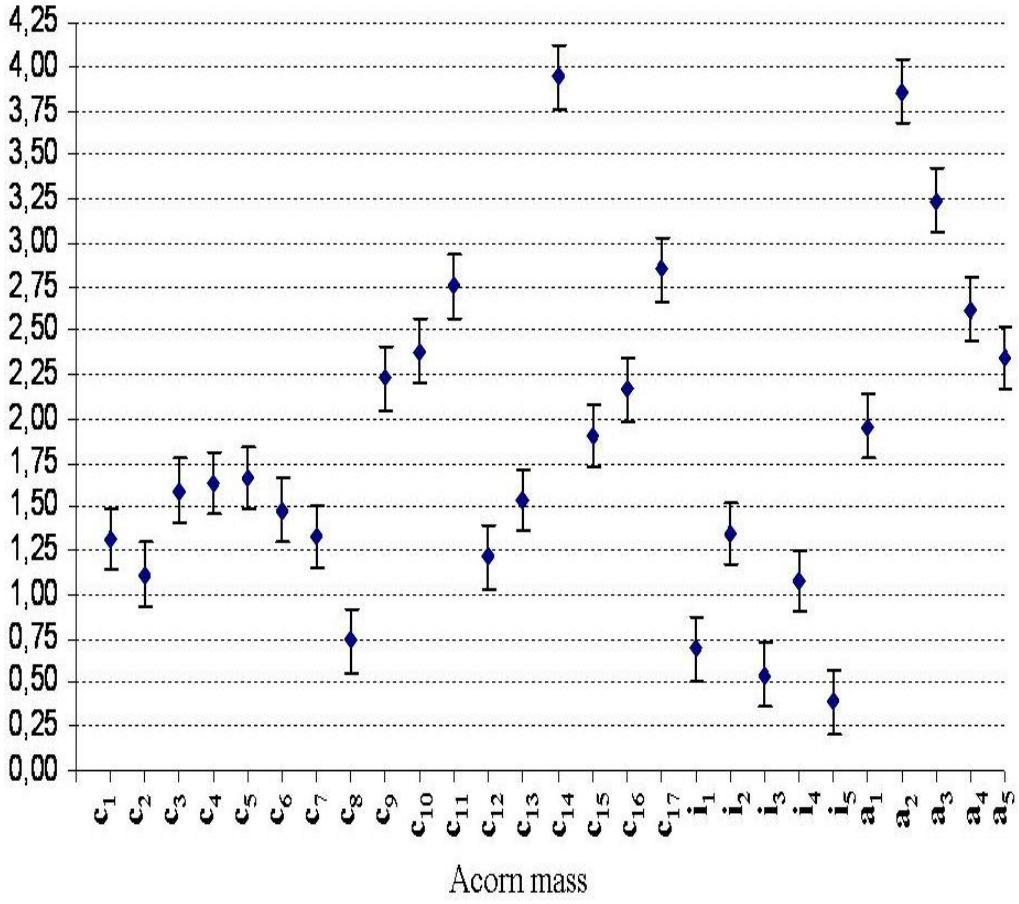

Figure 45- Means with standart error plots for acorn mass

Table 35- Scoring character states for acorn mass

Group number	Coding range	Character state
1	0–1,80	1
2	1,80–3,00	2
3	3,00– +∞	3

APPENDIX 2- CODES OF FRUIT CHARACTERS

Fruit characters	Nut length	Nut diameter	Nut scar diameter	Cupule outer diameter	Cupule thickness	Cupule scale length (max)	Cupule depth	Cupule length	Stalk length	Stalk thickness	Cupule length/nut length	Cupule depth/cupul length	Nut diameter/nut length	Cupule thickness/cupule outer diameter	Acorn mass
pop.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
c1	1	1	2	2	1	2	1	1	1	2	2	3	2	1	1
c2	1	1	2	1	1	2	1	1	1	1	2	2	1	1	1
c3	1	1	2	2	1	2	1	1	1	2	2	4	1	1	1
c4	1	1	2	2	1	2	1	1	1	2	1	3	1	1	1
c5	1	1	2	2	2	2	1	1	1	2	2	3	1	2	1
c6	1	1	2	2	1	3	2	1	1	2	2	3	2	1	1
c7	1	1	2	2	1	3	1	1	1	2	2	4	2	1	1
c8	1	1	1	1	1	3	1	1	1	1	2	3	2	1	1
c9	2	1	2	2	2	3	1	1	1	2	1	3	1	2	2
c10	2	1	2	3	2	3	2	2	1	2	2	2	1	2	2
c11	2	1	3	2	2	2	2	1	1	2	1	4	1	2	2
c12	1	1	2	2	2	3	1	1	1	2	2	3	2	2	1
c13	1	3	2	2	2	3	1	1	1	2	2	3	2	2	1
c14	2	2	2	3	2	2	3	2	1	3	1	3	1	2	3
c15	1	2	3	3	2	3	2	1	1	3	2	3	2	2	2
c16	1	2	3	3	2	4	3	2	1	3	2	3	2	3	2
c17	1	2	3	2	2	4	3	2	2	3	3	2	2	3	2
i1	1	1	1	1	1	1	1	1	2	1	2	2	1	1	1
i2	1	1	1	3	3	1	1	1	2	1	2	2	1	3	1
i3	1	1	1	1	1	1	1	1	2	1	2	1	1	1	1
i4	1	1	1	1	1	1	1	1	3	1	2	2	1	1	1
i5	1	1	1	1	1	1	1	1	2	1	3	1	2	3	1
a1	1	2	3	2	2	3	2	2	1	2	3	2	3	3	2
a2	2	2	3	3	2	3	2	2	1	2	1	2	1	2	3
a3	2	2	3	3	2	3	2	2	1	2	1	2	1	2	3
a4	1	2	3	3	2	3	2	2	1	2	2	2	2	2	2
a5	1	2	3	3	2	3	2	2	1	2	2	2	2	2	2

APPENDIX 3- VISUALIZATION OF RAPD PRODUCTS FOR EACH PRIMER IN ALL POPULATIONS WITH GEL ELECTROPHORESIS

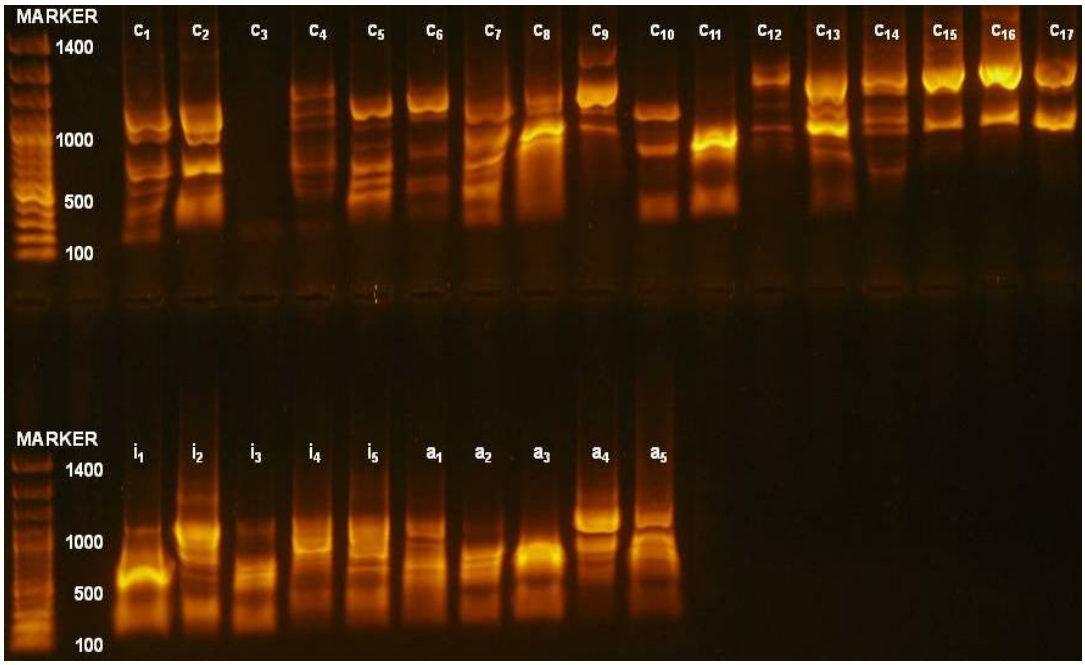

Figure 46- Visualization of RAPD products in all populations with OPX-04 primer

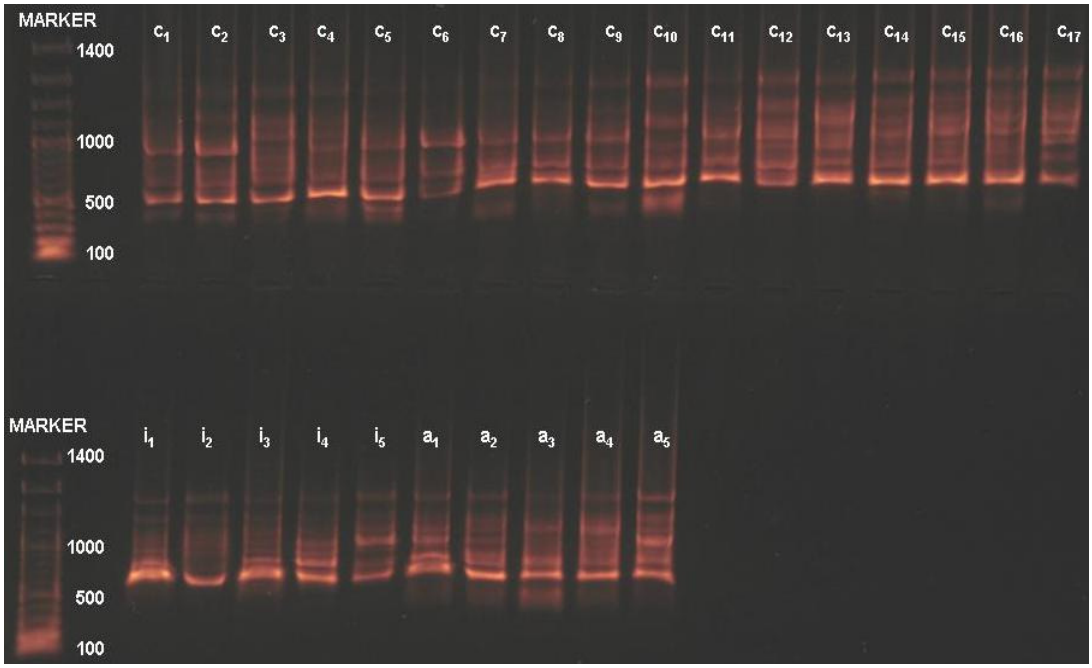

Figure 47- Visualization of RAPD products in all populations with OPC-09 primer

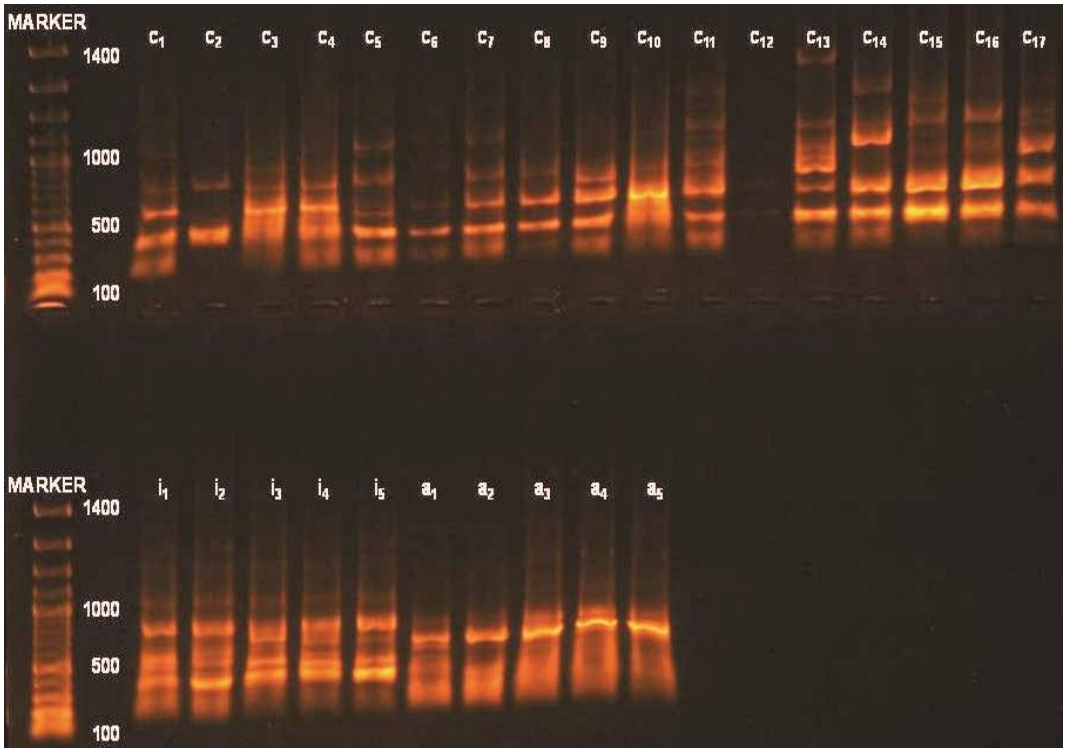

Figure 48- Visualization of RAPD products in all populations with OPA-08 primer

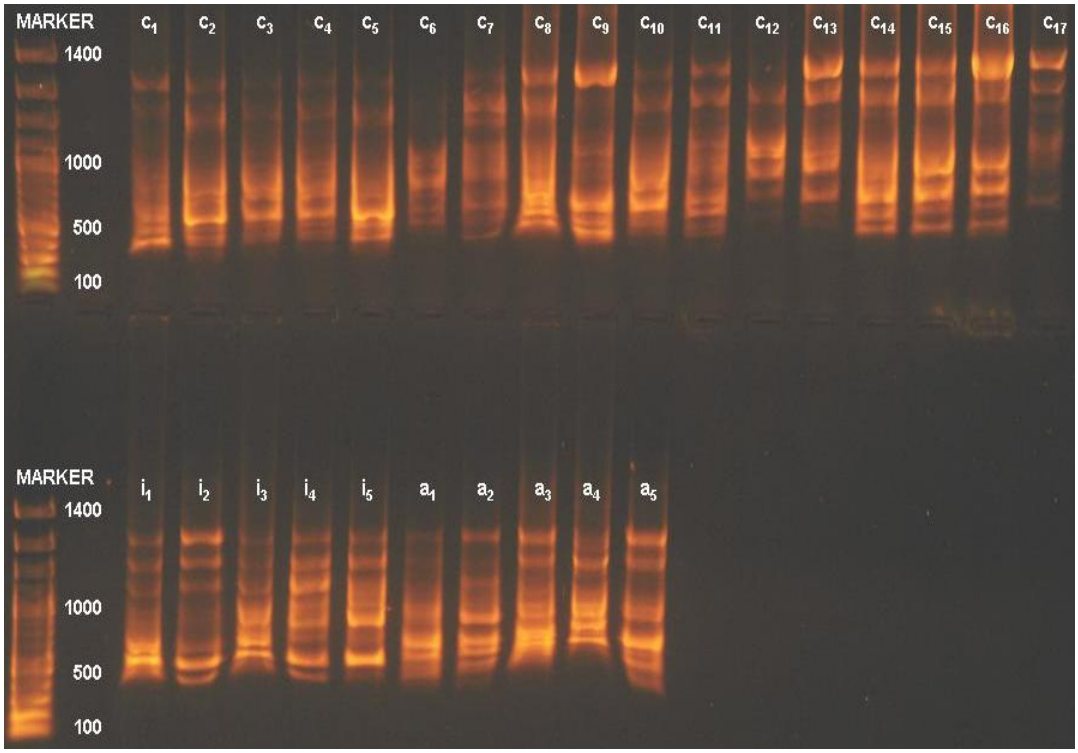

Figure 49- Visualization of RAPD products in all populations with OPS-09 primer

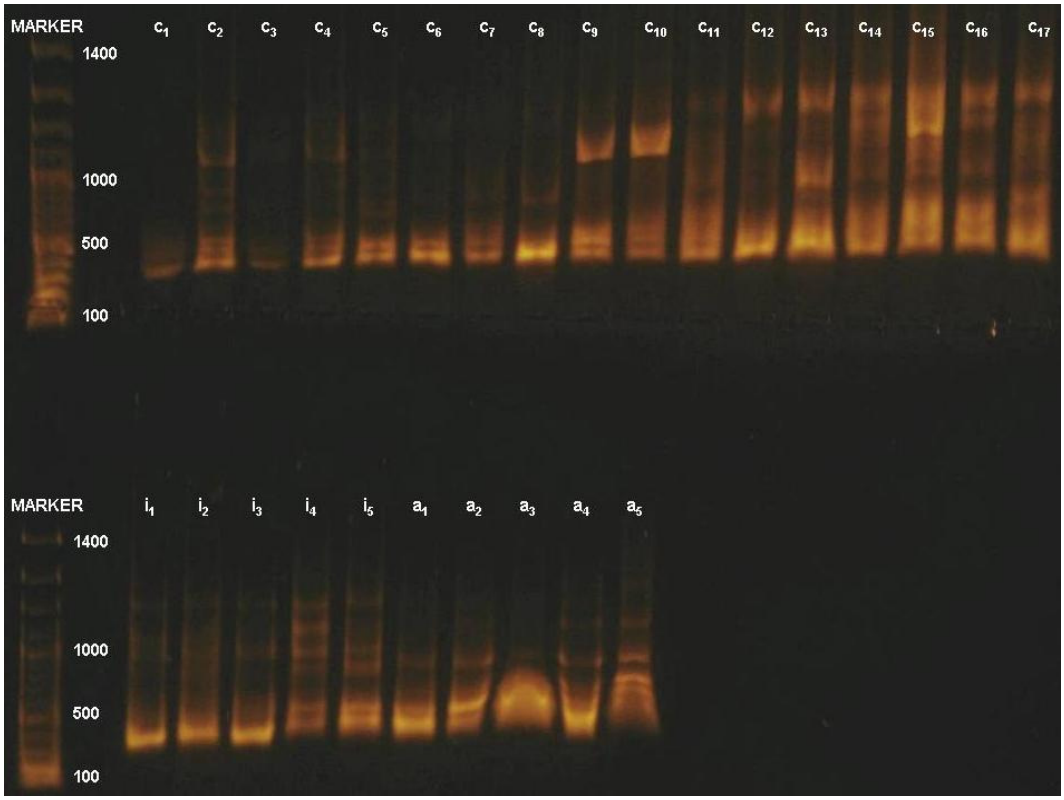

Figure 50- Visualization of RAPD products in all populations with OPA-09 primer

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Niğde University	2003-2006	M.Sc.	15.08.2006
Abant İzzet Baysal University	2006-2011	Ph.D.	12.09.2011

Abstracts:

- Yılmaz, A., Uslu, E. and Babaç, M.T. (2008) Karyological studies on four *Quercus* L. species in Turkey. Caryologia. 61(4): 397–401

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