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MORPHOLOGICAL VARIABILITY OF EVERGREEN OAKS (*QUERCUS*) IN TURKEY

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Abstract

The genus *Quercus* L. has a problematic taxonomy because of widespread hybridization among them. Evergreen *Quercus* contain three species in section *Ilex* Loudon namely, *Q. ilex* L., *Q. coccifera* L. and *Q. aucheri* Jaub. *et* Spach in Turkey. Here, two species, *Q. coccifera* and *Q. aucheri* are usually confused with each other. However, *Q. coccifera* and *Q. calliprinos* are accepted as different species but this subject is still controversial. Morphometric leaf and fruit variations of *Q. ilex*, *Q. coccifera* and *Q. aucheri* in 26 populations were measured for 25 characters. Variations within and among populations of species were detected by cluster analysis and principal component analysis. This study shows that populations of *Q. coccifera* from the south region of Turkey form a second group within *Q. coccifera*. Secondly, *Q. coccifera* show more similarity to *Q. aucheri* than *Q. ilex*, and finally there are two groups within *Q. coccifera*, which may be evaluated as *Q. coccifera* and *Q. calliprinos*.

Introduction

The genus *Quercus* L., popularly known as oaks shows highest morphological variations among species and populations (Hokanson *et al*., 1993; Kremer and Petit, 1993), especially its leaf characters are the most valuable in the classification and delimitation of species (Borazan and Babaç, 2003). The major reason for the phenotypic diversification of oaks is the high frequency of hybridization among species (Borazan andBabaç, 2003; Jensen, 1995).

Leaves are good indicators of putative hybridization and oaks can be easily identified by their leaves. The leaves of hybrid species have typically asymmetric shapes and are irregular (Jensen, 1995). Because of common interspecific hybridization in the genus *Quercus*, individuals that exhibit intermediate morphological characters can be seen widely. Sometimes, it is not possible to identify oak species due to high morphological variation. In this case, acorns are secondary important materials in oaks for classification and determination of hybridization (Jensen, 1988).

In Turkey three evergreen *Quercus* species exist, viz. *Q. aucheri, Q. coccifera* and *Q. calliprinos*. *Q. aucheri* is not very widely distributed, it only exists in the south western Anatolia region of Turkey and in the Greek islands like Rhodos. *Q. coccifera* is confused with *Q. calliprinos* Webb. (Toumı and Lumaret, 2001) because of small or medium shrub formation and acorn shape and evergreen nature. These two species may be evaluated within *Q. coccifera* as *Q. coccifera* subsp. *coccifera* and *Q. coccifera* subsp. *calliprinos* (Webb) Holmboe as two different species. This subject is still controversial (Salvatore and Paola, 1976; Toumı and Lumaret, 2001). *Q. ilex* the last member of *Ilex* section has two morphological types. These are the rotundifolia type containing small and round leaves and the ilex type containing big pointed leaves. The

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rotundifolia morphotype exists in North Africa and the interior region of Spain (Tutın *et al*., 1964). The ilex morphotype exists along the Atlantic coast of France. These two morphotypes are two different species (Tutın *et al*., 1964) or two subspecies (Saenz De Rıvas, 1967) or only two varieties (Maıre, 1961). Additionally, the presence of intermediate forms for these two morphotypes is reported in the Mediterranean region of France and in the north and east coasts of Spain. The aims of the present study were firstly to examine the morphological relationships among the populations sampled and their potential hybrids in Turkey, secondly to designate the status of confused two species, *Q. coccifera* and *Q. calliprinos*, and finally to evaluate and compare results provided from the leaf and acorn character.

Materials and Methods

The populations sampled are located in the 17 provinces that include the regions of North West, West, South and South West of Turkey. A total of 26 populations belonging to three species of *Ilex* section, namely *Quercus coccifera*, *Q. ilex* and *Q. aucheri* were collected. While 16 populations were designated to reveal variations within *Q. coccifera* (Table 1 and Fig. 1). *Q. aucheri* and *Q. ilex* were sampled in 5 populations owing to their distributions in a restricted region.

Table 1. Study populations with population number, location, coordinates and altitude (C = *Q. coccifera*, A = *Q. aucheri*, I = *Q. ilex*) *Q. coccifera* (C), *Q. aucheri* (A) *and Q. ilex* (I).

Pop. No.	*Q. coccifera* (C), *Q. aucheri* (A) and *Q. ilex* (I) Location	Coordinates N	Coordinates E	Altitude (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°40.350	027°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°33.069	029°03.150	940
C_{13}	Uşak-between Sivaslı-Uşak, 12 km. after Sivaslı	38°34.259	029°36.303	825
C_{14}	Gaziantep- between Yavuzeli-Araban	$37^0$22.975	$037^0$33.292	740
C_{15}	Kahramanmaraş- between k.maraş- göksun	$37^0$43.514	$036^0$40.038	1075
C_{16}	Hatay-between Kırıkhan-Hassa	$36^0$36.554	$036^0$23.591	350
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
I_1	Zonguldak-Alaplı, Sabırlı village	$41^0$08.901	$031^0$23.147	180
I_2	Zonguldak-between Alaplı-Düzce	41°08.443	031°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

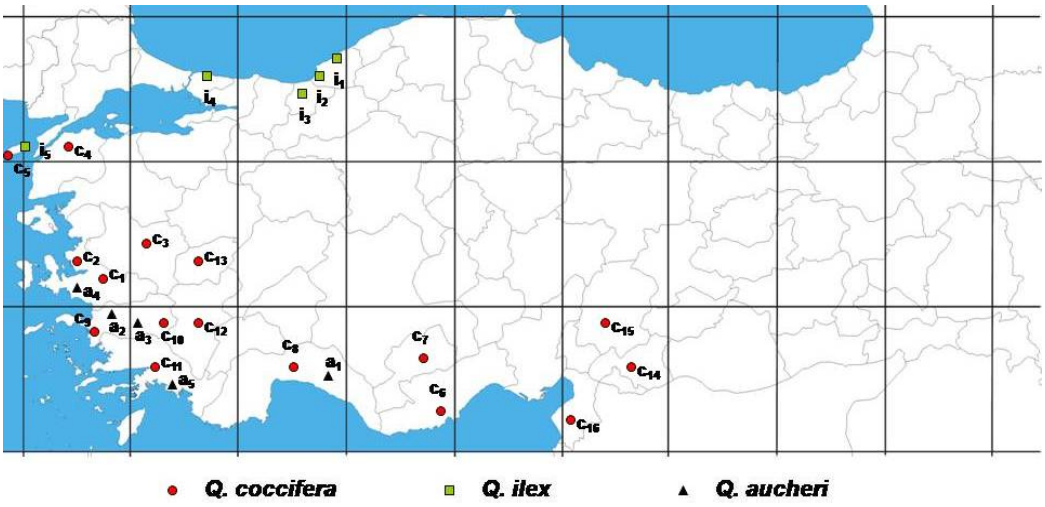

Fig. 1. Distribution of studied populations of *Q. coccifera*, *Q. ilex* and *Q. aucheri* in Turkey.

Leaf and fruit samples for identification and statistical analyses of each population were collected from 260 trees. In total, 2600 leaf and fruit materials were measured.All leaf samples were collected at the same height and location after leaf growth had stopped to avoid seasonal and positional variations as reported by Blue and Jensen (1988). Fruit samples were selected from mature acorns and cupules were also used as fruit characters.

Ten characters of leaves (Table 2 and Fig. 2) ad 15 characters from fruits (Table 3 and Fig. 3) were used. Most of the leaf characters were adopted from different sources (Bruschı *et al*., 2000; Kremer *et al*., 2002; Borazan and Babaç, 2003; Bruschı *et al.,* 2003; Gonzalez-Rodrıguez *et al.,* 2004; Ponton *et al*., 2004; Boratynskı *et al*., 2008).

Table 2. The leaf characters used in the morphological analysis.

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)

Table 3. The fruit characters used in the morphological analysis.

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

The fruit characters were also selected from literature (Nikoliç and Orloviç, 2002; Tılkı and Alptekin, 2005). Arithmetic means of all trees were calculated for each character. Then, means of the populations were calculated for each characters. Principal Component Analysis (PCA) and Cluster Analysis (CA) using Statistical version 8.0 were carried out for the analysis of variations in leaf and fruit samples.

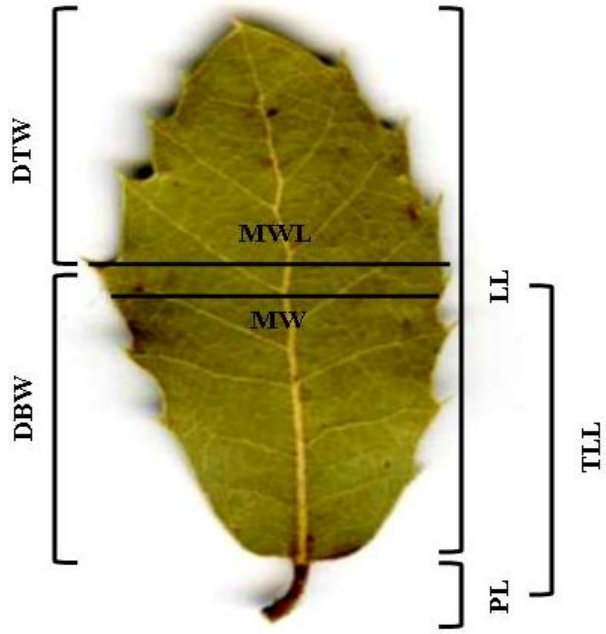

Fig. 2. Morphological leaf characters

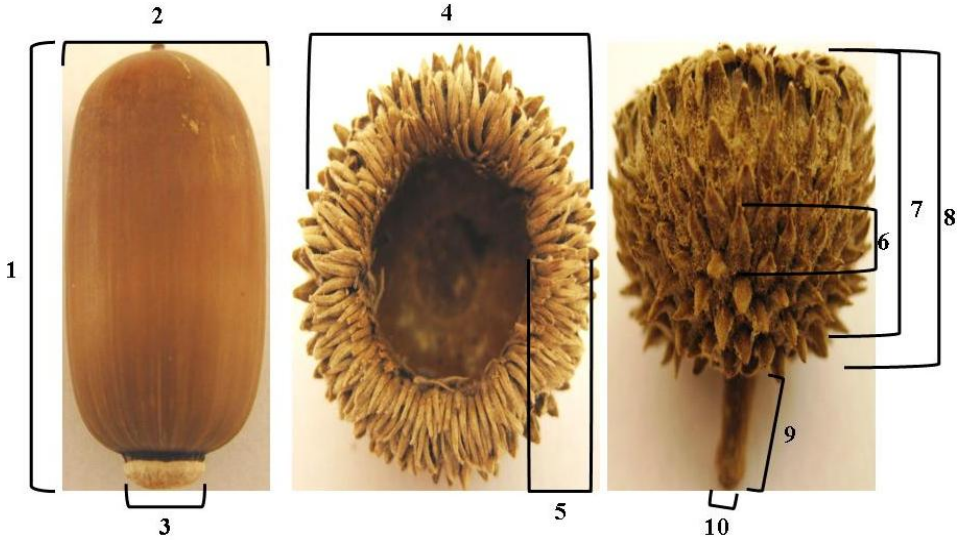

Fig. 3. Morphological fruit characters with character number.

Results

The UPGMA cluster analysis performed on the populations for leaf characters recognized two main groups one contains all populations of *Q. ilex* and another group consisted of 21 populations belonging to *Q. coccifera* and *Q. aucheri*. (Fig. 4) When the first group having the populations of *Q. ilex* is evaluated, it can be stated that populations of *Q. ilex* tend to form more morphologically discrete group than *Q. coccifera* and *Q. aucheri* populations and geographically close populations show more similarity like $İ_1$ and $İ_2$ populations (Fig. 4). Differences in geographical distribution are effective on species diversity. The biggest difference in the populations of *Q. ilex* is observed in $İ_5$ population and the locality of this population is an island in Aegean sea.

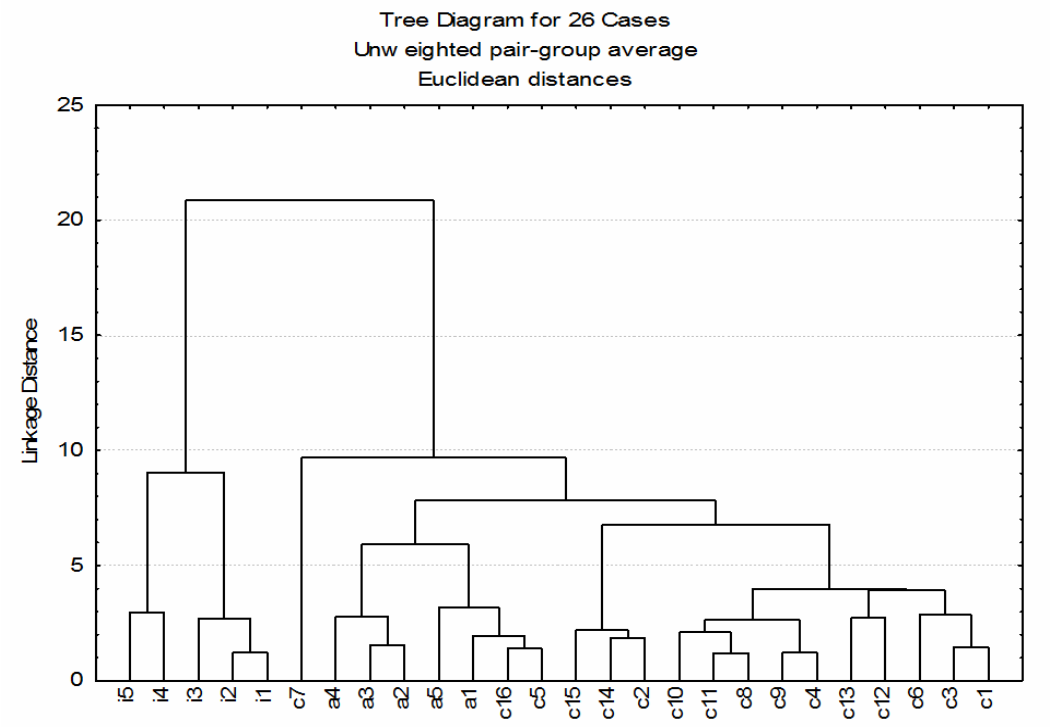

Fig. 4. Phenogram resulting from Cluster Analysis with UPGMA for the leaf materials.

The largest variation in second main group comprising of 21 populations is observed in C_7 population of *Q. coccifera* that occurs at high altitude (Table 1 and Fig. 4). Except C_7 population, the second main group is divided into two sub-groups, one consists of all populations of *Q. aucheri* and only C_{16} and C_5 populations of *Q. coccifera*, while other sub-group consists of complete populations of *Q. coccifera* (Fig. 4). The highest variation within this sub-group are observed in C_{14}, C_{15} and C_2 populations. Populations of *Q. aucheri* show the differences from populations of *Q. coccifera* within the second main group but this difference is not clear as in *Q. ilex*.

PCA results (Fig. 5) show the high similarity with CA (Fig. 4) results. PCA analyses clearly separate the *Q. ilex* populations from others. Similarly, two main groups are observed from PCA analysis. While one of these groups consists of populations belonging to *Q. ilex*, other two species are evaluated in the second group. C_{14}, C_{15} and C_{16} populations show the most differences within *Q. coccifera*. Results revealed that fruit characters, *Q. ilex*is is separated from the other two

species (Figs 6 & 7). CA results show clearly the presence of two main groups. The first main group consists of populations of *Q. ilex* and the second main group consists of the populations of *Q. coccifera* and *Q. aucheri*. This result shows the high similarity with the results of leaves.

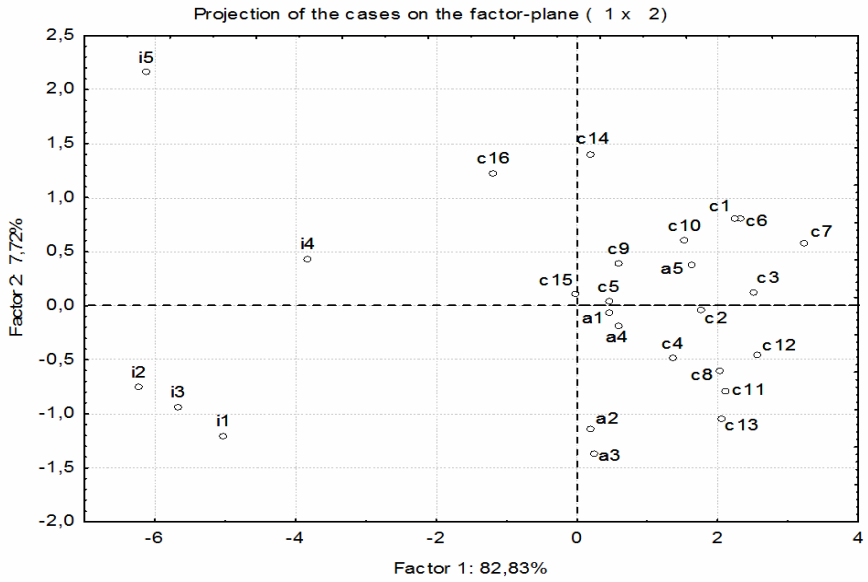

Fig. 5. Resulting projection of Principal Component Analysis for the leaf materials.

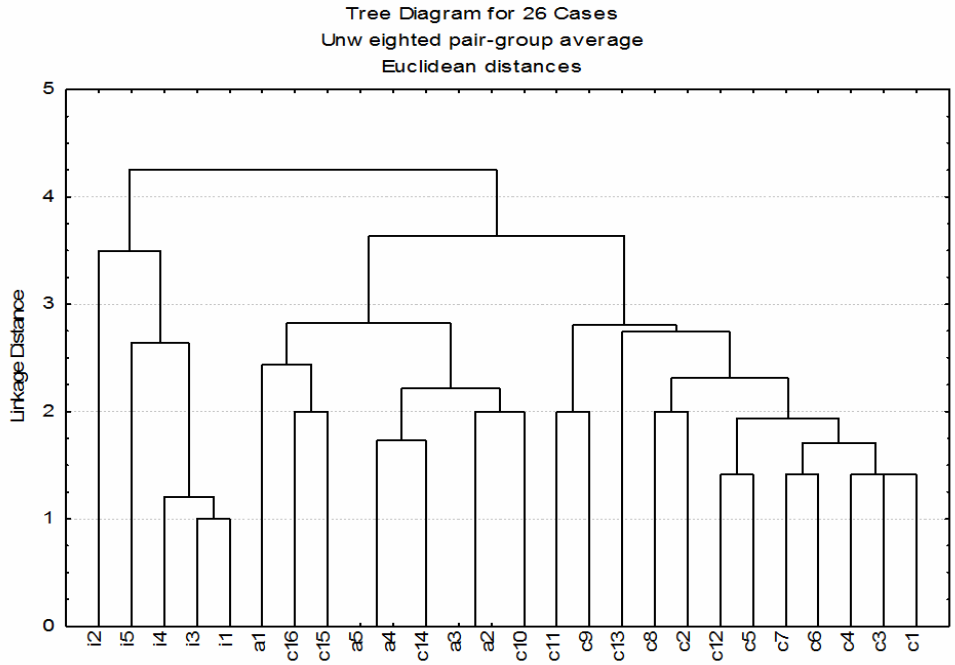

Fig. 6. Phenogram resulting from Cluster Analysis with UPGMA for the fruit materials.

Populations C_{10}, C_{14}, C_{15} and C_{16} form a discrete group with *Q. aucheri* in the CA graph (Fig. 6). However, remaining populations of *Q. coccifera* form other group (Fig. 6). Similar results showing differences among the species are observed in PCA (Fig. 7).

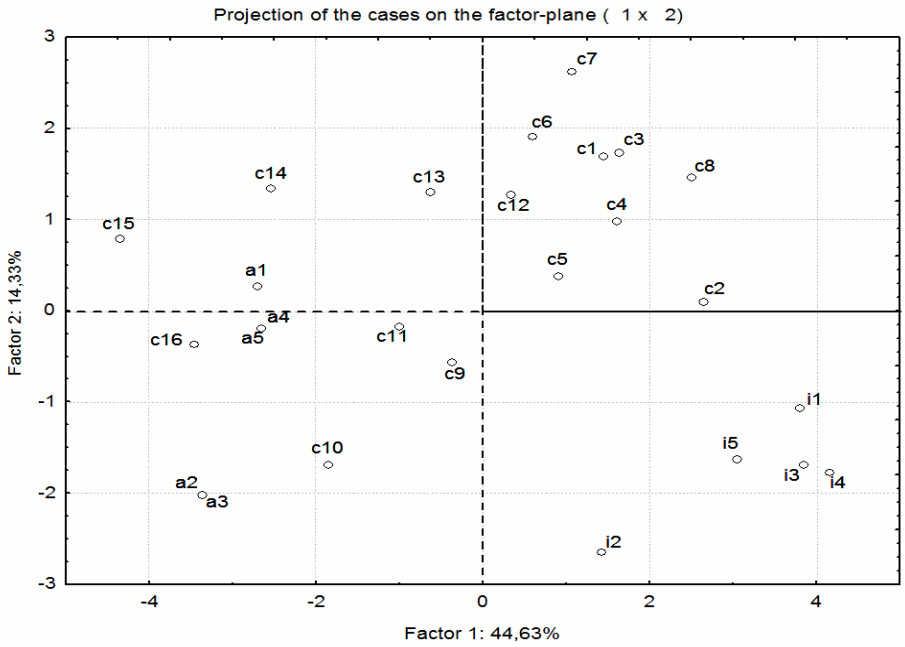

Fig. 7. Resulting projection of Principal Component Analysis for the fruit materials.

Discussion

The present study revealed that PCA and CA analyses could be used to solve taxonomic problems and to understand the relations among three species belonging to *Ilex* section of the genus *Quercus*. Leaf materials were generally used for the comparison of the oaks (Borazan and Babaç, 2003; Bruschı *et al.,* 2003; Ponton *et al.*, 2004; Gonzalez-Rodrıguez and Oyama, 2005; Franjıc *et al.,* 2006; Boratynski *et al*., 2008) but here, for the first time the fruit materials together with the leaf materials were examined in detail.

The results of morphometric studies provided the satisfactory findings for phenetic groupings of taxa in *Ilex* section. The most significant differences were found on the *Q. ilex* populations. This species was separated from the remaining species on the basis both leaf and fruit. However, *Q. coccifera* populations were grouped next to *Q. aucheri* populations. On the other hand, the results of *Q. coccifera* and *Q. aucheri* are not clearly separated from each other. Especially, due to similar leaf and fruit characters in both taxa, they showed introgression with each other in both CA and PCA plots (Figs 4-7). However, these results draw attention to the presence of a group away from *Q. coccifera*. The first group consists of the populations sampled from North West, West and South West regions of Turkey. The populations sampled from the south region of Turkey such as C_{14}, C_{15} and C_{16} was included into the second group showing the similarity to the populations of *Q. aucheri*. Result from the leaf and fruit studies supported these groupings. Similar results are also observed by Salvatore and Paola (1976), Toumı (1995),ToumıandLumaret (2001)and Yılmaz *et al.(*2013).

Geographically separation of *Q. coccifera* suggests that there are variation within this species. The restricted group of *Q.coccifera* located only in the south region of Turkey is geographically closer to Syria, Israel and Palestine. In Palestine there are two subspecies *Q. calliprinos* Webb. viz. *Q. calliprinos* sub sp. *coccifera* and *Q. calliprinos* sub sp. *calliprinos* (Zohary, 1966).

Our results suggested that the two groups showing geographical differences within *Q. coccifera* may be quite possibly strengthen the existence of two species as *Q. coccifera* and *Q. calliprinos* (Yılmaz *et al.,* 2013).

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