Phylogenetic Relationships in Quercus castaneifolia C.A. Mey. in Hyrcanian forests of Iran based on AFLP markers

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Abstract
Since the beginning of the Linnaean taxonomy, the classification within the genus Quercus has raised conflicting opinions, and more than 20 classifications were proposed. Quercus has a problematic taxonomy because of its immense size and wide distribution, heterophyllie, widespread hybridization between the infrageneric taxa and changes in morphological features. All oak species of Iran belong to the subgenus Quercus and two sections: sect. Quercus and sect. Cerris. Quercus castaneifolia belong to Cerris group and it is one of the most important species of Iran’s native oaks, distributed in the Hyrcanian forests. Pure and mixed stands of Q. castaneifolia cover about 6.5% of the Hyrcanian forests. This species shows morphological variations in leaf and acorn characteristics. The delimitation of Q. castaneifolia, number of its subspecies and varieties varies according to different authors. In the present study, we examined the use of AFLPs as an alternative for morphological markers and nuclear DNA sequences. AFLP is a DNA fingerprinting technique that generates large numbers of highly reproducible fragment markers with a genome-wide distribution. The objectives of the present study were (1) to reconstruct the phylogeny of Quercus castaneifolia C. A. Mey. While positioning and identifying hybrid taxa and cultivars, and (2) to evaluate the taxa proposed by other scientists. As a result of obtained information from genetic structure and cluster analysis in AFLP method, two taxa of Q. castaneifolia subsp. castaneifolia and Q. castaneifolia subsp. aitchisoniana from Cerris group are introduced in Hyrcanian region.

Key words: Iran, Quercus castaneifolia, AFLP, Hyrcanian forests, oak.

Introduction
Depending on the authors there are between 300 [9-18] and 600 [24] reported oak species. Since the beginning of the Linnaean taxonomy, the classification within the genus Quercus has raised conflicting opinions, from De Candolle (1868) to Nixon (1993), and more than 20 classifications were proposed. Disagreements are due either to the characters to be used for the classification, the infrageneric subdivisions adopted (sub genus or sections), and species delineation. There is so much variation within species that the concept of species has been questioned by several authors [3-39] and further complications in taxonomy are due to frequent interspecific hybridization. The genus Quercus is one of the most important groups of woody plants in many regions of the Northern Hemisphere. Oaks dominate various temperate, subtropical, and tropical forest types, and are also a major component of several chaparrals and scrub vegetation’s [1]. Quercus L. has a problematic taxonomy [4] because of its immense size and wide distribution [16-35], heterophyllie, widespread hybridization between the infrageneric taxa and changes in morphological features (e.g. [6-38]). Most of the information about the classification of Quercus has come from taxonomic studies where the emphasis is on foliar and fruit characteristics. Based on consideration of the species characteristics as a whole, most specialists have disagreed on the specific nomenclature for subgenera, species, varieties and forms [5-24-33-37]. Several oak species grow abundantly in the Zagros, Arasbaran and Hyrcanian forests displaying remarkable morphological variation. All oak species of Iran belong to the subgenus Quercus and two sections: sect. Quercus and sect. Cerris [20]. In the present study, we examined the use of AFLPs as an alternative for morphological markers and nuclear DNA sequences. AFLP is a DNA fingerprinting technique that generates large numbers of highly reproducible fragment markers with a genome-wide distribution. The

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technique is relatively fast and cost efficient and requires no prior knowledge of the genome [15-19-30-40]. Relative to morphological markers, AFLPs have the advantage that they are not under direct selection pressure, since most of the fragments represent noncoding parts of the genome [40]. AFLPs are more variable than chloroplast sequences [17]. Moreover, AFLPs represent both paternal and maternal lineages because they are almost entirely derived from the nuclear genome [2]. Compared to nuclear DNA sequences such as ITS, AFLPs have the advantage that they are more variable and that they are sampled across the entire genome rather than in a specific location [17]. However, AFLPs also have drawbacks that potentially may hamper their use as phylogenetic characters (reviewed in Koopman, 2005), most notably a possible lack of homology between fragments across taxa [2]. Several studies have shown that homology assignment between AFLP fragments decreases with increasing evolutionary distance between taxa [2-17]. Koopman (2005) contrasted AFLP variation with ITS sequence divergence in a large number of taxa and concluded that AFLPs are reliable phylogenetic markers for plant taxa with ITS sequences differing up to 30–35 nucleotides. A GenBank survey for the species in the present study revealed that ITS sequence differences among ingroup species ranged from 0 to 22 nucleotides, which is well within the range defined by Koopman (2005). Therefore, it is expected that the AFLP marker variation in our data set is a suitable indicator of Quercus relationships. Arens et al. (1998), Cervera et al. (2005), Ziegenhagen et al. (2008), and Smulders et al. (2008) demonstrated in poplar that the AFLP pattern of hybrid offspring contains bands of both parental species. Therefore, the comparison of AFLP patterns of taxa may serve to identify hybrids. The objectives of the present study were (1) to reconstruct the phylogeny of *Quercus castaneifolia* C. A. Mey. while positioning and identifying hybrid taxa and cultivars, and (2) to evaluate the taxa proposed by other scientists. Quercus species have covered huge parts of Iran’s forests [20]. The total forest coverage in Iran is 12 million hectares, which amounts to 8% of the total land area. About 1.8 million hectares of these forests are located in the north of Iran (i.e., the Hyrcanian Forests) belong to the end of the third geological era [13-14]. The Hyrcanian (from “Hyrcaemia”, the Greek form of an old Iranian word to describe the region of Gorgan) forests stretch in an arc along the southern shores of the Caspian Sea from the Talish region in Azerbaijan (at longitude 48°E) to Golestan national park in Iran (at longitude 56°E) and between latitudes 38°55′N in Azerbaijan Republic and 35°05′N in Iran [22]. Apart from this continuous belt located in provinces Ardebil, Gilan, Mazandaran and Golestan, there are some isolated forests, one in the west known as Arasbaran forest, located in East Azerbaijan and the forest isolates near Joozak located at 55 km west of Bojnurd, in Northern Khorassan province [1]. These forests are about 1000 kilometers in length and 20 to 70 kilometers in width. The Hyrcanian Forests consist of mixed broadleaf deciduous species [13-14]. *Quercus castaneifolia* belong to Cerris group and it is one of the most important species of Iran’s native oaks, distributed in the Hyrcanian Forests. As a main species of the Hyrcanian forests, it forms two important forest communities in this zone: Querco-Buxetum in the Caspian coast plains and Querco-Carpinetum in the lowlands (up to 700m a.s.l). With drier climate in the northeast of Iran, the later community gradually transforms into a Zelkovo-Quercetum community. *Quercus castaneifolia* is found in composition with other woody species on the upper altitudes. Pure and mixed stands of *Q. castaneifolia* cover about 6.5% of the Hyrcanian Forests [10]. This species shows morphological variations in leaf and acorn characteristics. The delimitation of *Q. castaneifolia*, number of its subspecies and varieties varies according to different authors. Various studies have been conducted on the Oaks of northern and north western Iran. However, these were based on Morphological, anatomical, or micro-morphological characteristics and have reported different results on *Quercus* number of taxa [5-7-11-20-21-29-31-32-36]. All scientists have agreed with the existence of the following 3 species in Hyrcanian and Arasbaran’s forests:

- *Quercus castaneifolia* C. A. Mey. subsp. *castaneifolia*
- *Q. macranthera* Fisch. and C.A.Mey. ex Hohen.
- *Q. petraea* L. ex Liebl. subsp. *iberica* (Steven) Krassiln

The following, discusses some of the current issues regarding this subject matter. At first, Meyer (1831) described *Q. castaneifolia* C. A. Mey. from the Caucasus and Hyrcanian Forests of Iran. Then Schwarz (1935) studied the herbarium specimens collected by Bornmüller from Bandar-Gaz forests in Golestan Province, and Rasht forests in Gilan province. He described a new species named *Q. sintenisiana* O. Schwarz, based on trichomes characteristics [32]. Three years later, Camus (1936-1938) described a new species named *Q. komarovii* A. Camus (1939) and reported two subspecies including: *Q. castaneifolia* C. A. Mey. subsp. *eucastaneifolia* A. Camus and *Q. castaneifolia* C. A. Mey. subsp. *aitchisoniana* A. Camus in his monograph of
world’s oaks [5]. The most complete study of Quercus in Iran was done by Djavanchir Khoie (1967) based on leaf and acorn morphology [7]. He classified the Iranian oaks into two subgenera; Quercus (lobed-leaved species) and Complanate Djav.-Khoie (dentate-leaved species), based on leaf venation. The subgenus Complanate was further divided into two sections; Oligandracee Djav.-Khoie and Polyandracee Djav.-Khoie based on number of stamens. According to this classification Q. castaneifolia belongs to section Oligandracee. Djavanchir Khoie treated Q. sintenisiana O. Schwarz and Q. castaneifolia C. A. Mey. subsp. eucastaneifolia A. Camus as synonyms of the type species but he accepted Q. castaneifolia C. A. Mey. subsp. aitchisoniana A. Camus and Q. komarovii A. Camus as separate taxa and introduced 8 taxa of Quercus castaneifolia from the Hyrcanian Forests as follows:

- Q. castaneifolia C. A. Mey. subsp. Incurvata Djav.-Khoie
- Q. castaneifolia C. A. Mey. subsp. subrotundata Djav.-Khoie
- Q. castaneifolia C. A. Mey. subsp. triangularis Djav.-Khoie
- Q. castaneifolia C. A. Mey. subsp. Undulata Djav.-Khoie
- Q. castaneifolia C. A. Mey. subsp. aitchisoniana A. Camus
- Q. castaneifolia C. A. Mey. subsp. castaneifolia var. castaneifolia
- Q. castaneifolia C. A. Mey. subsp. castaneifolia var. ellipsoidalis Djav.-Khoie
- Q. castaneifolia C. A. Mey. subsp. castaneifolia var. minuta Djav.-Khoie

Menitsky in Flora Iranica (1971), categorized Iran’s native oaks into subgenus: Quercus, and two sections: Quercus and Cerris. He placed dentate leaved species in section Cerris and lobed leaved species in section Quercus. According to his classification, Q. macranthera and Q. petrea subsp. iberica were considered in section Quercus and Q. castaneifolia was considered in section Cerris and all infraspecific taxa recognized by Djavanchir Khoie and Camus were treated as its synonyms. He recognized only Q. castaneifolia C. A. Mey. subsp. castaneifolia from Iran [20]. In 2011 for the first time in Iran, a research was corrode out by Panahi to study the micro-morphological foliar characteristics and type of trichomes (including trichomes type, trichomes length, type of epicuticular waxes, the type and shape of the stomata) and pollen (including pollen morphology and exine ornamentations). As a result, 11 taxa of Hyrcanian forests were accepted to have largely similarities to accepted taxa by Djavanchir Khoie (1967) in the region [25-26-27-28-29]. Considering economical and ecological importance of Oaks in Alborz Mountains, and given the fact that their current classification is mainly based upon their morphological characteristics, resulting in lot of disagreements on the existing classification, it is highly warranted to employ novel techniques and approaches such as molecular studies to resolve taxonomic problems of the genus.

![Figure 1](image)

Figure 1. Type localities of published species of Quercus (each number indicates one Taxon):
1. Q. castaneifolia subsp. castaneifolia var. castaneifolia (1)
2. Q. castaneifolia subsp. castaneifolia var. castaneifolia (2)
3. Q. castaneifolia subsp. castaneifolia var. castaneifolia (3)
4. Q. castaneifolia subsp. castaneifolia var. ellipsoidalis
5. Q. castaneifolia subsp. castaneifolia var. minuta
6. Q. castaneifolia subsp. aitchisoniana
7. Q. castaneifolia subsp. incurvata
8. Q. castaneifolia subsp. subrotundata
9. Q. castaneifolia subsp. triangularis
10. Q. castaneifolia subsp. undulata
11. Q. sintenisiana
Materials and Methods

Plant samples
Plant sampling has been done from type location of all identified species and sub-species in Hyrcanian province including Golestan, Mazandaran, Gilan, Ardabil and East Azerbaijan and total of 104 herbarium specimen samples have been obtained. In addition to herbarium specimen preparation, from five populations of each herbarium specimen, fresh leaves were collected and kept in 50 CC falcon tubes, filled with Silica Gel, for the purpose of drying them. The leaves were then used as a DNA extraction source. The plant specimen was identified in Department of Biology, Science and Research Branch, Tehran Islamic Azad University, Iran and a voucher specimen of the plant with number 14527 - 14542 is deposited in the Avicenna herbarium of this department (IAUH) (Table 2). Based on morphological evidence and with the help of identification keys, obtained from previous studies, 11 taxa were distincted from Quercus castaneifolia taxa of Hyrcanian province, 8 of which were based on previous studies [5-7-20-26-28-31-32-36] and 3 taxon referring to Q. castaneifolia subsp. castaneifolia var. castaneifolia based on observed morphological differences in various regions, were distincted to measure their relationships based on molecular evidence.

DNA extractions and AFLP genotyping
Total DNA was extracted following a modified CTAB protocol of Doyle and Doyle (1990) [8] using the DNeasy Plant Mini kit (Qiagen, Germany). The AFLP assay (Vos et al. 1995) was performed after digestion/ligation with the 6-bp cutting enzyme EcoRI and the 4-bp cutting enzyme MseI, followed by a two-step polymerase chain reaction (PCR) amplification protocol (Arens et al. 1998) with the modification of using fluorescence-labeled primers [40].

Table 1. List of name and primer pair sequence used on selective PCR reaction

<table>
<thead>
<tr>
<th>No</th>
<th>Reverse primer (Nonfluorescent)</th>
<th>Name of Reverse primer</th>
<th>Forward primer (Fluorescent)</th>
<th>Name of Forward primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5'-GATGAGTCCTGAGTAACGG-3'</td>
<td>M-57</td>
<td>5'HEX-GACTGCGTACCAATTCACT-3' Green</td>
<td>E-38</td>
</tr>
<tr>
<td>2</td>
<td>5'-GATGAGTCCTGAGTAACCT-3'</td>
<td>M-54</td>
<td>5'FAM-GACTGCGTACCAATTCAATG-3' Blue</td>
<td>E-45</td>
</tr>
<tr>
<td>3</td>
<td>5'-GATGAGTCCTGAGTACAGA-3'</td>
<td>M-55</td>
<td>5'NED-GACTGCGTACCAATTAGC-3' Yellow</td>
<td>E-40</td>
</tr>
</tbody>
</table>

PCR selective step has been done with the use of Fluorescent Primer (Biolegio BV, the Netherlands). In this step only one of the used selective primers on each PCR reaction has been Fluorescent (E). Fluorescence-labeling E primers happened just at 5' end and according the table 1. M series selective primers have been used without any changes and fluorescence-labeling. Finally selective PCR reactions have been done with 3 pairs of primers. When reactions are done, triple product of reactions for each sample with ratio of 2.5, 3.75 and 3.75 respectively mixed for blue, green and yellow colors. Then for each sample 2 micro liter of mixed product of reactions have been mixed with 7.75 microliter formamide (Applied Biosystems) and 0.25 microliter Gene Scan ROX Standard red color (Applied Biosystems) and injected to ABI 3730 Sequencer. After 4-5 hours results have been executed as chromatogram file.

AFLP data analysis

A) Genetic Structure analysis
In analysis of Genetic Structure, Structure 2.3.4 software were used according to bayesian statistical methods. Search parameters have been defined as following:
Model: Admixed model
Alpha: infer Alpha, same Alpha for all populations, initial: 1, max: 10
Allele frequency: independent
Value of Lambda: 10
Q-hat: print Q-hat, frequency of Metropolis update for Q: 10
For finding real number of populations (K-haplotypes), Evanno et al. (2005) method is used this step repeated 20 times, assuming population`s numbers are 1-10 with total cycles of 60000 MCMC then first 10000 cycles are ignored. then delta K index calculated with following method according to average LN P(D) (approximate probability of K) result for each assumption of population`s number.
Delta K = \[\frac{L' (K)}{\text{St.-Dev.} [L(K)]}\]
L (K) = mean of 20 values of LN P(D)
L' (K) = L (K) - L (K-1)
L" (K) = L' (K + 1) - L' (K)  

after calculation of population's real number (K), MCMC analysis have been done one more time assuming 1250000 cycles and first 500000 cycles are ignored.

B) Cluster Analysis

Multivariate statistical algorithm is one of the best classification ways of Genetic pools and genetic diversity analysis among taxa of population with huge samples among which cluster analysis could be mentioned. Cluster analysis refers to a group of multivariate techniques whose primary purpose is to group individuals or objects based on the characteristic they possess, so that individuals with similar descriptions are mathematically gathered into the same cluster the resulting clusters of individuals should then exhibit high internal (within cluster) heterogeneity. Thus, if the classification is successful, individuals within a cluster shall be farther apart Distance based clustering methods can be categorized into two groups: hierarchical and nonhierarchical. Hierarchical clustering method is more commonly employed in analysis genetic diversity in crop science. The most similar individuals are first grouped and these initial groups are merged according to their similarities. Among various agglomerative hierarchical methods, the UPGMA (Unweighted Paired Group Method Using Arithmetic Average) is the most commonly adopted clustering algorithm fallowed by the Ward’s minimum variance method [12]. The distance-based methods, distance matrix are analyzed as an input and output is resulted as tree or graphically. At final categorizing, taxa similarities in each group are more than their similarities between the groups. For data cluster analysis, softwares such as SPSS or NT-SYS could be used so in this research SPSS software has been used. One of the most important aspects of cluster analysis is to determine eligible and acceptable cluster. In fact accuracy of the result depends on cutting area of dendrogram for finding real groups.

Table 2. List of Quercus castaneifolia taxa in this study. The taxonomic framework for suprageneric categories follows Djavanchir Khoie, K. (1967) & Schwarz, O. (1935). Accession numbers in the GenBank are listed in the last column.

<table>
<thead>
<tr>
<th>Subgenus</th>
<th>Species, Subspecies, Varieties</th>
<th>Collection data</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Altitude</th>
<th>Accession number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerris group</td>
<td>Q. castaneifolia subsp. castaneifolia var. castaneifolia (1)</td>
<td>Mazandaran Prov., Between Noor and Slahedin kola (IAUH-14527)</td>
<td>N: 36° 34' 10&quot;</td>
<td>E: 51° 53' 41&quot;</td>
<td>0 m</td>
<td>KM579605</td>
</tr>
<tr>
<td></td>
<td>Q. castaneifolia subsp. castaneifolia var. castaneifolia (2)</td>
<td>Golestan Prov., Shir abad, Shir abad Waterfall (IAUH-14529)</td>
<td>N: 36° 58' 31&quot;</td>
<td>E: 55° 00' 49&quot;</td>
<td>118 m</td>
<td>KM583450</td>
</tr>
<tr>
<td></td>
<td>Q. castaneifolia subsp. castaneifolia var. castaneifolia (3)</td>
<td>Gilan Prov., Between Sowme'eh Sara and Taher gorab (IAUH-14528)</td>
<td>N: 37° 21' 29&quot;</td>
<td>E: 49° 44' 02&quot;</td>
<td>107 m</td>
<td>KM583451</td>
</tr>
<tr>
<td></td>
<td>Q. castaneifolia subsp. castaneifolia var. castaneifolia</td>
<td>Golestan Prov., Ali abad katoul, Kabood val Waterfall (IAUH-14530)</td>
<td>N: 36° 52' 07&quot;</td>
<td>E: 54° 53' 09&quot;</td>
<td>407 m</td>
<td>KM586065</td>
</tr>
<tr>
<td></td>
<td>Q. castaneifolia subsp. castaneifolia var. incurvata</td>
<td>Mazandaran Prov., Zirab, Laajim village (IAUH-14530)</td>
<td>N: 36° 14' 47&quot;</td>
<td>E: 53° 07' 08&quot;</td>
<td>890 m</td>
<td>KM586072</td>
</tr>
<tr>
<td></td>
<td>Q. castaneifolia subsp. incurvata</td>
<td>Mazandaran Prov., Tonekabon, Sehezar, Falak deh (IAUH-14532)</td>
<td>N: 36° 35' 5&quot;</td>
<td>E: 50° 50' 09&quot;</td>
<td>854 m</td>
<td>KM586062</td>
</tr>
<tr>
<td></td>
<td>Q. castaneifolia subsp. incurvata</td>
<td>Mazandaran Prov., Chalus, Dasht e nazir (IAUH-14535)</td>
<td>N: 36° 25' 01&quot;</td>
<td>E: 51° 24' 51&quot;</td>
<td>945 m</td>
<td>KM586066</td>
</tr>
<tr>
<td></td>
<td>Q. castaneifolia subsp. subrotundata</td>
<td>Gilan Prov., Between Astara and Harandan (IAUH-14531)</td>
<td>N: 38° 08' 18&quot;</td>
<td>E: 48° 53' 17&quot;</td>
<td>13 m</td>
<td>KM586067</td>
</tr>
<tr>
<td></td>
<td>Q. castaneifolia subsp. triangularis</td>
<td>Ardabil Prov., Fandoghlou forest (IAUH-14536)</td>
<td>N: 38° 23' 42&quot;</td>
<td>E: 48° 32' 43&quot;</td>
<td>1442 m</td>
<td>KM586073</td>
</tr>
<tr>
<td></td>
<td>Q. castaneifolia subsp. undulata</td>
<td>Ardabil Prov., Between Astara and Ardabil, Heyran (IAUH-14534)</td>
<td>N: 38° 24' 09&quot;</td>
<td>E: 48° 41' 34&quot;</td>
<td>281 m</td>
<td>KM586069</td>
</tr>
<tr>
<td></td>
<td>Q. sintenisiana</td>
<td>Mazandaran Prov., Sisangan forest (IAUH-14539)</td>
<td>N: 36° 34' 29&quot;</td>
<td>E: 51° 48' 40&quot;</td>
<td>34 m</td>
<td>KM586069</td>
</tr>
</tbody>
</table>
Results and Discussion

A) Genetic structure:
After 20 times repetition of MCMC (Markov chain Monte Carlo) process, for 1-9 assumed population, obtained results of Structure 2.3.4 software are as following:

<table>
<thead>
<tr>
<th>Delta K</th>
<th>L'' (K)</th>
<th>L' (K)</th>
<th>St.-Dev. [L(K)]</th>
<th>repetition</th>
<th>L (K)</th>
<th>K</th>
</tr>
</thead>
<tbody>
<tr>
<td>-91.60530191</td>
<td>105.74</td>
<td>-105.74</td>
<td>102.985</td>
<td>20</td>
<td>1.1543</td>
<td>2</td>
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<tr>
<td>-0.586125006</td>
<td>1.16</td>
<td>-1.16</td>
<td>-2.755</td>
<td>20</td>
<td>1.9791</td>
<td>3</td>
</tr>
<tr>
<td>-0.166436025</td>
<td>0.445</td>
<td>-0.445</td>
<td>-3.915</td>
<td>20</td>
<td>2.6737</td>
<td>4</td>
</tr>
<tr>
<td>-1.871390793</td>
<td>6.935</td>
<td>-6.935</td>
<td>-4.36</td>
<td>20</td>
<td>3.7058</td>
<td>5</td>
</tr>
<tr>
<td>-4.027290762</td>
<td>108.79</td>
<td>-108.79</td>
<td>-11.295</td>
<td>20</td>
<td>27.0132</td>
<td>6</td>
</tr>
<tr>
<td>0.179429482</td>
<td>99.825</td>
<td>99.825</td>
<td>-120.085</td>
<td>20</td>
<td>556.3467</td>
<td>7</td>
</tr>
<tr>
<td>0.239528072</td>
<td>153.435</td>
<td>153.435</td>
<td>-20.26</td>
<td>20</td>
<td>640.5721</td>
<td>8</td>
</tr>
<tr>
<td>-5.619743688</td>
<td>133.175</td>
<td>-133.175</td>
<td>133.175</td>
<td>20</td>
<td>23.6977</td>
<td>9</td>
</tr>
</tbody>
</table>

As per table 3, maximum amount of Delta K happens when population number is assumed equal to 2 then MCMC analysis has been done by assuming total number of 1250000 cycles and ignoring first 500000 cycles of K=2 on total data. Figure 2 representing taxa genetic structure according to their arrangement on analysis.

AFLP markers on population studies shows that despite to high variety in one population and visible differences of taxa, variety between populations and vegetation areas is very small. Based on the results, only two different genotypes on population and taxa detected and their Hybridization in Mazandaran is more than other areas. Two different Genotypes are as follows: 1- Q. castaneifolia subsp. castaneifolia and 2- Q. castaneifolia subsp. aitchisoniana. Genetic diversity on samples belonged to areas of Golestan, Semnan, Gilan, Ardabil and East Azerbaijan province was less than Mazandaran (Figure 3).
Figure 3. Relation between genetic structure and vegetation areas of taxa and population of studied Quercus castaneifolia on Hyrcanian forests. Scientific names according to Djavanchir:

1- Q. castaneifolia subsp. castaneifolia var. castaneifolia (2) 2- Q. castaneifolia subsp. castaneifolia var. castaneifolia (1) 3- Q. castaneifolia subsp. castaneifolia var. castaneifolia (3) 4- Q. castaneifolia subsp. aitchisoniana 5- Q. castaneifolia subsp. subrotundata 6- Q. castaneifolia subsp. undulata 9- Q. sintenisiana 13- Q. castaneifolia subsp. castaneifolia var. minuta 14- Q. castaneifolia subsp. castaneifolia var. ellipsoidalis.

B) Cluster analysis of AFLP data

As figure 4 shows, dendrogram contains 33 taxon of 9 populations; in discussed dendrogram all taxa in distance of more than 25% dissimilitude are included in the same group. Also on numerous cases, taxa of one population got separated from a population and getting them mixed with different populations which it proves equality of DNA and genetic structure of different populations. in 7 population out of 9 of them in discussing group, their population are not placing together in dendrogram (populations 1-2-3-4-5-13-14) while based on previous studies there are Morphological and anatomical differences on them and it caused their classification to be on sub species division. As well taken into attention that different population were collected from far distances of each other (6 different provinces). All case study samples in dendrogram of figure 4, are for taxa of Cerris Group which according to previous studies, from this group only related taxa to Quercus castaneifolia species are existing in Hyrcanian areas. As result of cluster analysis of data in AFLP method, it’s not able to certainly differ pure population as a separate taxon from other taxon. As what is shown in dendrogram, related taxa to Q. castaneifolia subsp. aitchisoniana in distance of more than 9% dissimilitude are placed in the same group and will be separated from other taxa on top of dendrogram. As a result of obtained information from genetic structure and cluster analysis in AFLP method, two taxa of Q. castaneifolia subsp. castaneifolia and Q. castaneifolia subsp. aitchisoniana from Cerris group are introduced in Hyrcanian region which result is meeting the obtained result of ITS Comparing the sequences in this taxa by author and also Camus classification (1993). Morphological differences observed in this group do not follow a consistent process and they can be caused by polymorphism, hybridization, ecological and any other factors which it can’t be considered as a stable diagnostic features to separate taxa and only Q. castaneifolia subsp. aitchisoniana Compared to the type specimen, have very short nut (14ml), multiplied scale couple, impressively wider towards base and non-curved toward the end, which overall can be considered as a separate sub-species of this region (Figure 5).
Figure 4: Dendrogram of the genetic diversity of first group (Cerris group) comes from oak population of Hyrcanian region using AFLP markers’ data. Number on the left represents population number and next one indicates number of each sample which population are as following: 1- Q. castaneifolia subsp. castaneifolia var. castaneifolia 2- Q. castaneifolia subsp. castaneifolia var. castaneifolia (i) 3- Q. castaneifolia subsp. castaneifolia var. castaneifolia (3) 4- Q. castaneifolia subsp. aitchisoniana 5- Q. castaneifolia subsp. subrotundata 6- Q. castaneifolia subsp. undulata 9- Q. sintenisiana 13- Q. castaneifolia subsp. castaneifolia var. minuta 14- Q. castaneifolia subsp. castaneifolia var. ellipsoidalis.
Figure 5: Herbarium specimen prepared in this research (left picture: Q. castaneifolia subsp. aitchisoniana and Right picture: Q. castaneifolia subsp. castaneifolia). Voucher specimen with number 14532 and 14527 is deposited in the Avicenna herbarium (IAUH).

References