Caspian Journal of Environmental Sciences
Online ISSN: 1735-3866
Print ISSN: 1735-3033

Molecular diversity and phylogenetic analysis of Azerbaijan oaks (*Quercus* spp.) revealed by RAPD markers

Gullu Aliyeva*[1], Javid Ojaghi[2], Samira Rustamova[3]

1. Institute of Dendrology, Azerbaijan National Academy of Sciences, Baku, Azerbaijan
2. Khazar University, Life Sciences department, Azerbaijan
3. Institute of Molecular Biology & Biotechnologies, Azerbaijan National Academy of Sciences, Baku, Azerbaijan

** Corresponding author's E-mail: bio890@mail.ru*

ABSTRACT

Although Azerbaijan is a center of natural distribution and diversity of genus *Quercus* around the world, less published data can be found about PCR-based genetic diversity in prevalent oak accessions. The present study aims at finding molecular variation among the *Quercus* species using RAPD markers. In this study, genomic fingerprinting in five Azerbaijan oak species (*Quercus* spp.) was carried out using 10 RAPD markers. The result showed that a total of 113 DNA fragments were amplified, of those, 91 bands were polymorphic. The highest PIC, EMR and MI values was observed in the primers OPD-05, OPB-01 and OPB-03. The genetic similarity among the genotypes examined ranged from 0.333 to 0.818 with an average of 0.498. Species-specific DNA fragments were found in all species examined. Based on the cluster analysis using UPGMA method and Jaccard's similarity index, all the examined oak species fell into three main groups and principal coordinate analysis (PCoA) supported the clustering result. High genetic diversity was found in the tested genetic germplasm of oak species. The results of this study would be very useful for oak improvement program.

Key words*: Quercus, genetic variation, molecular marker, polymorphism.*

INTRODUCTION

Quercus is an expansive genus consisting of over 500 species from all corners of the globe. Oaks have long been considered among the strongest and noblest of all woody plants, and exhibit an impressive range of ecological, industrial and horticultural merit (Dosmann & Aiello 2013). Since Darwinian time, botanists have used oaks as a model genus for studying evolutionary processes and speciation. Oaks have characteristics such as high levels of phenotypic plasticity, interspecific gene flow and genetic variation, which significantly contributed to the genesis of several hundreds of species, subspecies and ecotypes (Neophytou *et al.* 2010). Indeed, these characteristics influence the biological success of the genus *Quercus*. However, these properties also pose difficulties in classification of this genus, estimating genetic differentiation among species and genetic architecture of populations (Ardi *et al.* 2012).

The Republic of Azerbaijan has a very rich flora and over 4,500 species of higher plants have been registered in the country. Due to the unique climate in Azerbaijan, the flora is much richer in the number of species than the flora of the other republics of the South Caucasus and around 65% of the Caucasus' plant diversity, including approximately 200 endemics of Azerbaijan and 950 endemics of Caucasus were recorded in Azerbaijan (Solomon *et al.* 2014). *Quercus* is one of the most important woody genera of the Northern hemisphere and considered as one of the main

Caspian J. Environ. Sci. Vol. 19 No. 3 pp. 457~468
DOI: 10.22124/CJES.2021.4932
Received: April 01. 2021 Accepted: June 22. 2021
Article type: Research

forest tree species in Azerbaijan (Menitsky 2005). The oak tree has a special symbolic, ecological and economical value in Azerbaijan.

It is generally recognized that plant genetic diversity changes in time and space. The extent and distribution of genetic diversity in a plant species depends on its evolution and breeding system, ecological and geographical factors, past bottlenecks, and often by many human factors (Arekhi *et al.* 2010; Larsary *et al.* 2021). Much of the large amount of diversity of a species may be found within individual populations, or partitioned among a number of different populations (Ramanatha Rao & Hodgkin 2002).

Protection and conservation of high value forest genetic resources requires information on the patterns of genetic variation among and within populations (Saenz-Romero *et al.* 2003; Ostadhashemi *et al*. 2014; Attarod *et al.* 2017). This aim can be achieved by molecular genetic markers that have provided a powerful and reliable new tool for breeders to search for new sources of genetic diversity, polymorphisms and relationship of populations of different origins (Baig *et al.* 2008). The assessment of genetic diversity is one of the key steps in any plant breeding program as the classical methods become less efficient for the identification of different varieties. Molecular as well as morphological characterization need to be conducted to clarify the relationships between genotypes. These data should then be applied as inputs to the breeding process of the desired traits (Zeinali *et al.* 2009). Molecular approaches collectively represent a potential gold mine of important information that can be applied as an efficient tool for effective characterization of germplasm (Kumar *et al.* 2018). DNA fingerprinting techniques have been widely used to analyze the genetic variation and to differentiate of species or populations in plant conservation management (Abdessemed *et al.* 2015; Miyashita *et al.* 2015; Patel *et al.* 2018; Kumar & Agrawal 2019). RAPD is a relatively recent technique and has been widely used for the estimation of molecular diversity in various crops due to its low cost, rapid and easy assay, and needs a small amount of plant material with prior sequence information (Hamouda 2019). The RAPD has been used with success to identify and determine relationships at the species, population, and cultivar levels in many plant species (Naik *et al.* 2010; Singh *et al.* 2019; Samaha *et al.* 2019; Antić *et al.* 2020).

There is a lack of information on the genetic diversity and population structure of the *Quercus* species in Azerbaijan. So, the present study was carried out using RAPD markers to find molecular variation among oak species collected from some regions of Azerbaijan Republic. In the present study, the RAPD fingerprinting technique was employed to understand the population genetic structure of Azerbaijan *Quercus* species. It is a part of a larger study on the ecological, morphological, and molecular characterization of these five species in Azerbaijan (Aliyeva *et al.* 2020 a, b; Aliyeva *et al*. 2021; Aliyeva 2021).

MATERIALS AND METHODS

Plant sampling

Six genotypes from four species which distributed in the flora of Azerbaijan (*Q. pedunculiflora* (Baku), *Q. pedunculiflora* (Absheron), *Q. macrhantera* (Goygol), *Q. iberica* (Ismailli), *Q. castaneifolia* (Absheron), *Q. castaneifolia* (Lankaran) and a genotype from a species introduced in the Azerbaijan flora, i.e. *Q. ilex* (Absheron) were selected for the isolating DNA (Fig. 1). Each tree was originated from seed, with 50 to 120 years of age. For each tree, three to five young intact leaves were collected for the molecular analysis. The leaves were immediately frozen in liquid nitrogen and then transferred to a -80°C freezer until further analyses.

Total genomic DNA isolation

DNA extraction was performed by the CTAB method with some modifications (Murray & Thompson 1980). Fresh plant tissue in a leaf fragment form was grinded in liquid nitrogen and suspended in 1000 µL CTAB extraction buffer (100 mM Tris-HCl, pH 8.0; 20 mM EDTA, pH 8.0; 1.4 mM NaCl; 40 mM β-mercaptoethanol), preheated in a water bath to 60 °C. Homogenization was completed by intense shaking in vortex. Then 400 µL chloroform (99.8%) was added to each tube and the contents were gently mixed. Thereafter, the test tubes were placed in a water bath and incubated for 10 min at 60 °C. After incubation, the tubes were centrifuged in a tabletop Eppendorf type centrifuge (15000 g) for 10 min at room temperature. Then the supernatant was carefully isolated (taking care not to capture the particles of precipitate) and transferred to a clean Eppendorf-type 1.5 mL-test tubes and 600 µL cold isopropanol was

Caspian J. Environ. Sci. Vol. 19 No. 3 pp. 457~468 Received: April 01. 2021 Accepted: June 22. 2021
DOI: 10.22124/CJES.2021.4932 Article type: Research

added to it, mixed well and left at room temperature for 3-5 min. At this stage the disperse DNA precipitate can be observed. The tube contents were centrifuged at room temperature in a tabletop Eppendorf-type centrifuge (15000 g) for 10 min. The precipitate was washed several times with 70% ethanol, dried in a thermostat at 56 °C for 5 min and dissolved in TE buffer (10 mM Tris-HCl, pH 8; 1 mM EDTA). Samples were left overnight in a refrigerator at 4 °C to completely dissolve the DNA in the buffer.

Determination of DNA concentration

After dissolution of the DNA, its concentration was determined by optical density (OD) at λ = 260 nm using the ULTROSPEC 3300 PRO ("AMERSHAM", USA) spectrophotometer. Purity of genomic DNA was determined by A260/A280 absorbance ratio. Quality of the DNA was verified by activity of extracted DNA samples in a 0.8% agarose gel stained with 10 mg mL^{-1} ethidium bromide in 1 × TBE buffer (Tris base, Boric acid, EDTA). The gel was developed and photographed under UV light using Gel Documentation System (UVITEK, UK).

Fig. 1. Oak samples collected areas: Baku, Absheron, Lankaran, Goygol, Qabala and Ismayilli.

DNA amplification

Polymerase chain reaction (PCR) using RAPD markers was performed by Williams method (Williams *et al*., 1990). DNA amplification was performed in a reaction mix of 25 µL volume containing 10 x buffer, 20 ng genomic DNA, 0.2 µM primer, 200 µM each: dATP, dCTP, dGTP and dTTP, 2.5 mM $MgCl_2$, and 0.2 unit Taq-polymerase in incubation buffer. 10 oligonucleotide primers were used for RAPD analysis. Nucleotide sequences of the primers are shown in Table 1. PCR was performed in the Applied Biosystems 2720 Thermal Cycler (Singapore) thermocycler in the following conditions: 1cycle: 4 min at 94 ºC; 10 cycles: 1 min at 94 ºC, 1 min at 36 ºC and 1 min at 72 ºC; 35 cycles: 1 min at 94 ºC, 1 min at 36ºC, 1 min at 72 ºC; the final elongation cycle was performed at 72°C for 15 min and stored at 4°C.

The reaction products were separated by electrophoresis in a 1.5% agarose gel in the HR-2025-High Resolution (IBI SCIENTIFIC, USA) horizontal electrophoresis device with addition of ethidium bromide. Gels were documnted using Gel Documentation System (*UVITEK*). A 100 to 3000 bp DNA ladder was included in all gels as a reference, to estimate the size of the amplified fragments. To assess the reproducibility of PCR products, the DNA from each species was first amplified independently three times with each primer, and the presence/absence of each band was then scored in all individuals.

Caspian J. Environ. Sci. Vol. 19 No. 3 pp. 457~468
DOI: 10.22124/CJES.2021.4932

Received: April 01. 2021 Accepted: June 22. 2021
Article type: Research

Data scoring and statistical analysis

Out of the twenty ISSR and twenty-two SCoT primers tested, eight ISSR and nine SCoT primers produced clear and polymorphic bands, then were chosen for further analysis. Banding patterns produced by eight ISSR and nine SCoT markers were scored for absence (0) and presence (1) of bands. Initially, by observing the banding patterns produced by different ISSR and SCoT primers, total number of bands, polymorphic bands and percentage polymorphism were obtained. Further, potential of these molecular markers for estimation of genetic variability was assessed by measuring polymorphism information content (PIC), effective multiplex ratio (EMR), marker index (MI) and resolving power (RP). PIC values were calculated using the formula PIC = 1- $\sum p_i^2$, where pi is the frequency of the i^{th} allele (Smith *et al.* 1997). Marker index (MI) is the primer capacity to detect polymorphic loci among different genotypes and was calculated as EMR X PIC, where, EMR is the product of number of polymorphic loci and fraction of polymorphic loci.

Table 1. List of the RAPD primers used in the present study.

Primer	Sequence	C+G (%)
OPB-01	5´-GTTTCGCTCC-3´	60
OPB-02	5´-TGATCCCTGG-3´	60
OPB-03	5´-CATCCCCCTG-3´	70
OPB-04	5´-GGACTGGAGT-3´	60
OPB-05	5´-TGCGCCCTTC-3´	70
OPD-05	5´-TGAGCGGACA-3´	60
OPD-08	5´-GTGTGCCCCA-3´	70
OPD-11	5´-AGCGCCATTG-3´	60
OPA-03	5´-AGTCAGCCAC-3´	60
OPA-04	5´-AATCGGGCTG-3´	60

RESULTS AND DISCUSSION

Polymorphism level and marker efficiency

The greater the genetic diversity of a species and more complex its genetic background, the stronger its evolutionary potential and ability to withstand adversity, and the more easily it will expand its distribution range and adapt to new environments (Zhao *et al*. 2014). Therefore, the knowledge of the genetic diversity estimated from different genetic marker systems provides useful information to address breeding programs and germplasm resource management. Molecular markers are indispensable tools for measuring the diversity of plant species. Low assay cost, affordable hardware, throughput, convenience and ease of assay development and automation are important factors when choosing a technology (Arif *et al*., 2010). By developing modern biotechnology, many experiments have been successfully used for the evaluation of genetic diversity, identification, and phylogeny analyses of plant species by many different authors in molecular level (Ojaghi & Akhundova 2010; Patzak *et al.* 2012; Chen *et al.* 2013; Alikhani *et al.* 2014; Luo *et al.* 2014; Sorkheh *et al.* 2016; Gürcan *et al.* 2016; Salayeva *et al.* 2016; Luo *et al.* 2019).

So far there has been no report on the comparison of genetic diversity and phylogenetic analysis of *Quercus* spp. using molecular markers in Azerbaijan. However, several experiments have been run throughout the world by authors on oaks species by molecular marker systems in genome level (Lopez-Aljorna *et al.* 2007; Khadivi-Khub *et al.* 2015;

Caspian J. Environ. Sci. Vol. 19 No. 3 pp. 457~468 Received: April 01. 2021 Accepted: June 22. 2021
DOI: 10.22124/CJES.2021.4932 Article type: Research

Neophytou *et al.* 2015; Shi *et al.* 2017; Ashley *et al.* 2018; Degen *et al.* 2019). RAPD is one of the common genetic markers, used for population genetic analysis, pedigree analysis and taxonomic discrimination (Volis *et al.* 2001; Subramanyam *et al.* 2009; Yeasmin *et al.* 2015). Several authors have demonstrated that the RAPD–PCR method is a powerful tool in the assessment of discriminating differences at intra- and inter-population levels in oaks species (Yakovlev & Kleinschmidt 2002; Schiller *et al.* 2004; Fernandes *et al.*, 2011; Aykut *et al.* 2013; Jurkšienė *et al.* 2019). In the present study fifteen RAPD primers were tested with three oak genotypes as DNA templates. All primers produced amplification products, and only primers showing clear and reproducible band patterns were selected for further analyses. Thus, only ten primers were chosen for species identification and phylogenetic analyses (Table 2). We used a total of 7 *Quercus* species from different region of Azerbaijan. The banding patterns and polymorphism of primer OPB-02 are shown in Fig. 2. RAPD-PCR analyses showed that 10 random primers generated 113 distinct bands and 91 of them were polymorphic (Table 1). The main area of distribution of fragments was located in the range of 250-3500 bp (Fig. 2). The total number of amplified DNA bands for each individual ranged from 7 (OPA-04 and OPB-05) to 16 (OPD-11) with the average number of 11.3 bands per primer. The highest number of polymorphic bands was obtained by primer OPB-02, while the minimum number by OPB-05. On average, 9.1 polymorphic bands were obtained. The highest level of polymorphism (92.31%) was found by primer OPD-05, while the lowest polymorphism (57.14%) by OPB-05.

In the present study, high PIC value was observed for OPD-05, OPB-03 and OPB-01 at 0.96, 0.952 and 0.948, respectively. The low PIC value (0.48) was obtained by primer OPB-05, with an average of 0.752 per primer. High level of genetic diversity obtained in the collection and proved to be sufficiently genetically diverse and therefore the studied Azerbaijan oak genotypes would be useful to breeders for the development of new variety. Alikhani et al. (2014) reported that the average polymorphism information content for ISSR, IRAP and SCoT markers were 0.30, 0.32 and 0.38, respectively. PIC is considered as the better parameter for measuring genetic diversity than the number of alleles, taking into account the relative frequencies of each allele (Laborda *et al.* 2005). The highest effective multiplex ratio (EMR) and marker index (MI) were observed by OPD-05, OPB-01 and OPB-03. However, the lowest was observed by OPB-05, with an average EMR of 8.39 and an average MI of 6.66 per primer. The obtained high EMR and MI values in this study, indicated that the used RAPD markers are useful for the assessment of genetic variation and phylogenetic analysis among oak populations.

Species-Specific Markers

Several amplified RAPD bands showed fixed frequencies in a particular species and these can be used as species-specific markers. These unique DNA fragments can be developed into the sequence characterized amplified region (SCAR) marker after cloning and sequencing. Therefore, the SCAR marker can be used for marker-assisted selection program as a quick, efficient and reliable tool to identify among oak species. In the present study, 22 RAPD fragments were identified as species-specific markers between the examined 10 primers in all the oak species. However, we did not detect any species-specific fragments by OPB-04 and OPD-08. The maximum number of unique bands with 5 was revealed by primer OPD-11 in three oak species. Species-specific RAPD markers were also reported by Golan-Goldhirsh *et al.* (2004) in *Pistacia*, Dnyaneshwar *et al.* (2006) in *Phyllanthus emblica*, Sen *et al.* (2010) in *Piperaceae*, and Patel *et al.* (2015) in *Ocimum*. The details of species-specific bands detected in all oak species are shown in Table 3.

Similarity matrices based on RAPD markers

The binary data matrix generated by the amplified fragments of oak species in the RAPD-PCR analyses was used for the computations of Jaccard similarity index for every pairwise comparison of the genotypes (Table 4). The estimated similarity ranged from 0.333 to 0.818. Alikhani et al. (2014) in assessment of genetic diversity of *Quercus brantii* based on combining of SCoT, IRAP and ISSR data reported the Jaccard genetic similarity in the range of 0.23 and 0.76. In the present study, the results revealed that *Q. pedunculiflora* (Baku) and *Q. pedunculiflora* (Absheron) genotypes were closely related, having the highest genetic identity (0.818), while he lowest values belonged to *Q. ilex* (Absheron) and *Q. castaneifolia (*Absheron) with 0.333, then with 0.364 belonged to *Q. pedunculiflora* (Baku) *Q. castaneifolia (*Absheron), *Q. pedunculiflora* (Absheron) and *Q. castaneifolia (*Absheron), *Q. iberica* (Ismayilli) and *Q.*

Caspian J. Environ. Sci. Vol. 19 No. 3 pp. 457~468
DOI: 10.22124/CJES.2021.4932

Received: April 01. 2021 Accepted: June 22. 2021
Article type: Research

ilex (Absheron), which were the most distant genotypes. The average similarity across all the oak species was found out to be 0.498, showing that all genotypes were highly diverse to each other. These results suggest that efficiency of markers with these values can be employed in a breeding program such that the genotypes with the lowest genetic similarities could be selected as parents to improve the oak genotype.

Genetic relationships among genotypes

Development of desired genotypes requires assessment of genetic variability as the basis of breeding. Hybridization among selected genotypes may create a new gene pool with specific traits (Talas Oğraş *et al.* 2017). Hybridization can be carried out among the genotypes that belong to distance clusters. Thus, a wide range of segregants could be obtained for desired characters (Aminul Islam *et al.* 2016). It is known that the crossing of highly statistically distant genotypes from the clusters leads to variations among the segregants (Talas Oğraş *et al.* 2017).

Table 2. Details of RAPD primers and band details in *Quercus* spp.

Primer	Sequence (5'-3')	TAB	NPB	PPB	PIC	EMR	MI
OPA-03	AGTCAGCCAC	8	5	62.50	0.62	5.9	3.66
OPA-04	AATCGGGCTG	7	6	85.71	0.76	8.7	6.61
OPB-01	GTTTCGCTCC	10	9	90.00	0.948	11.4	10.81
OPB-02	TGATCCCTGG	15	13	86.67	0.79	9.8	7.74
OPB-03	CATCCCCCTG	11	10	90.90	0.952	10.8	10.28
OPB-04	GGACTGGAGT	12	9	75.00	0.69	6.8	4.69
OPB-05	TGCGCCCTTC	7	4	57.14	0.48	4.8	2.30
OPD-05	TGAGCGGACA	13	12	92.31	0.96	11.8	11.32
OPD-08	GTGTGCCCCA	14	11	78.57	0.68	7.8	5.31
OPD-11	AGCGCCATTG	16	12	75.00	0.64	6.1	3.90
Total	-	113	91	-	-	-	-
Minimum	-	7	4	57.14	0.48	4.8	2.30
Maximum	-	16	13	92.31	0.96	11.8	11.32
Mean	-	11.3	9.1	79.38	0.752	8.39	6.66

TAB: Total amplified bands; NPB: Number of polymorphic bands; PPB: Percentage of polymorphic bands; PIC: Polymorphism information content; EMR: Effective multiplex ratio; MI; Marker index.

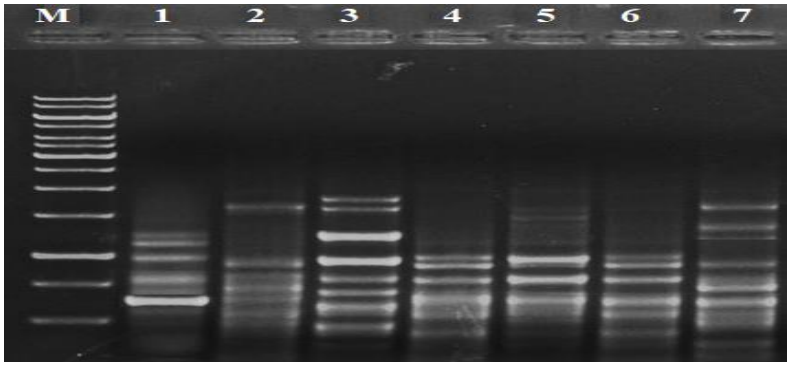

Fig. 2. RAPD pattern of different species of oaks by primer OPB-02. Lane 1: *Q. pedunculiflora* (Baku), lane 2: *Q. pedunculiflora* (Absheron), lane 3: *Q. macrhantera* (Goygol), lane 4: *Q. iberica* (Ismayilli), lane 5: *Q. ilex* (Absheron), lane 6: *Q. castaneifolia (*Absheron), lane 7: *Q. castaneifolia* (Hirkan) and M-molecular weight marker.

Caspian J. Environ. Sci. Vol. 19 No. 3 pp. 457~468 Received: April 01. 2021 Accepted: June 22. 2021
DOI: 10.22124/CJES.2021.4932 Article type: Research

Cluster analysis is a powerful and useful method for the evaluation of genetic relationships and phylogenetic studies (Randi & Lucchini 2002). In the present study, the dendrogram of the seven oak species by RAPD markers data was constructed based on Jaccard similarity coefficient using the UPGMA method (Fig. 3).

Table 3. Species-specific RAPD markers in each oak species.

Primer	Fragment No.	Size (bp)	Species
OPA-03	4	1200	*Q. macrhantera*
	6	650	*Q. macrhantera*
OPA-04	3	950	*Q. castaneifolia*
	5	750	*Q. castaneifolia*
	7	500	*Q. iberica*
OBP-01	2	2500	*Q. macrhantera*
	4	700	*Q. pedunculiflora*
	8	600	*Q. pedunculiflora*
	10	350	*Q. ilex*
OPB-02	14	450	*Q. iberica*
OPB-03	2	1500	*Q. castaneifolia*
	4	950	*Q. ilex*
OPB-05	7	450	*Q. macrhantera*
OPD-05	3	2500	*Q. iberica*
	5	1500	*Q. castaneifolia*
	10	550	*Q. iberica*
	12	450	*Q. macrhantera*
OPD-11	3	2000	*Q. pedunculiflora*
	7	950	*Q. iberica*
	9	750	*Q. macrhantera*
	13	450	*Q. iberica*
	15	350	*Q. pedunculiflora*

The results of the dendrogram showed that the examined *Quercus* species could be completely divided into three main groups based on the values of the similarity coefficient from 0.333 to 0.818. The first cluster includes only *Q. castaneifolia* from Absheron region of Azerbaijan, which indicate genetically distinct from other examined *Quercus* species. The second cluster consist of *Q. iberica* from Ismayilli, *Q. pedunculiflora* belonged to Absheron region, *Q. castaneifolia* from Hirkan region and *Q. pedunculiflora* from Baku region. In this group the lowest similarity was found between *Q. iberica* and *Q. castaneifolia* with 0.521 value. However, the highest similarity was revealed among *Q. pedunculiflora* from Absheron and Baku regions with 0.818 similarity index. In this clustering *Q. ilex* (Absheron)

Caspian J. Environ. Sci. Vol. 19 No. 3 pp. 457~468 Received: April 01. 2021 Accepted: June 22. 2021
DOI: 10.22124/CJES.2021.4932 Article type: Research

and *Q. macrhantera* (Goygol) are resided in the third cluster with similarity of 0.625. Clustering of *Quercus* species suggests that all the species examined are distinctively differentiated. Several authors have reported clear cut clustering in dendrograms based on RAPD investigates, demonstrating variations among different species (Ramanayake *et al.* 2007; Arghavani *et al.* 2010; Thomas *et al.* 2010; Mohammed *et al.* 2012). In our study, the cophenetic correlation coefficient between the similarity matrix and the UPGMA dendrogram was r = 0.861. The cophenetic values higher than 0.80 indicate a strong fit of dendrograms (Rohlf 2000).

Table 4. Jaccard similarity index of the oak species based on RAPD markers

No.	G1	G2	G3	G4	G5	G6	G7
G1	1						
G2	0.818	1					
G3	0.600	0.455	1				
G4	0.636	0.636	0.556	1			
G5	0.545	0.545	0.625	0.364	1		
G6	0.364	0.364	0.571	0.625	0.333	1	
G7	0.560	0.560	0.476	0.522	0.571	0.381	1

Genetic relationships among oak species were also investigated by principal coordinate analysis (PCoA). The data generated from RAPD markers were subjected to PCoA performed based on Jaccard coefficient of similarity. In the 2D plot of PCoA derived from the RAPD genotyping data, it can be observed that main three groups were formed on the plot (Fig. 4). The PCoA revealed the first two most informative coordinates with eigenvalues 0.328 and 0.222, respectively, which together accounted for 73.48% of the total genetic variation. The results of the PCoA confirmed the results of UPGMA clustering. According to results of PCoA and cluster analysis, it may be concluded that RAPD analysis revealed substantial polymorphism in the examined Azerbaijan oak species. The technique may be used to obtain reasonably precise information on the genetic relationship among genotypes. Such information may be useful for selecting the diverse parents and monitoring the genetic diversity periodically in the breeder's working collection of oak species.

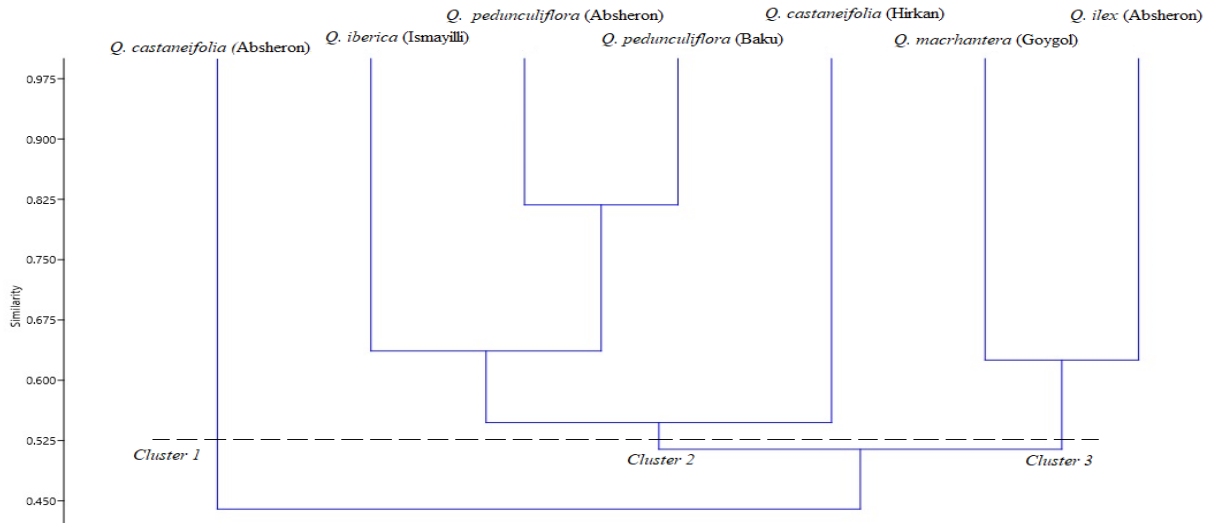

Fig. 3. UPGMA dendrogram of RAPD analyses of *Quercus* species based on Jaccard coefficient.

Caspian J. Environ. Sci. Vol. 19 No. 3 pp. 457~468
DOI: 10.22124/CJES.2021.4932

Received: April 01. 2021 Accepted: June 22. 2021
Article type: Research

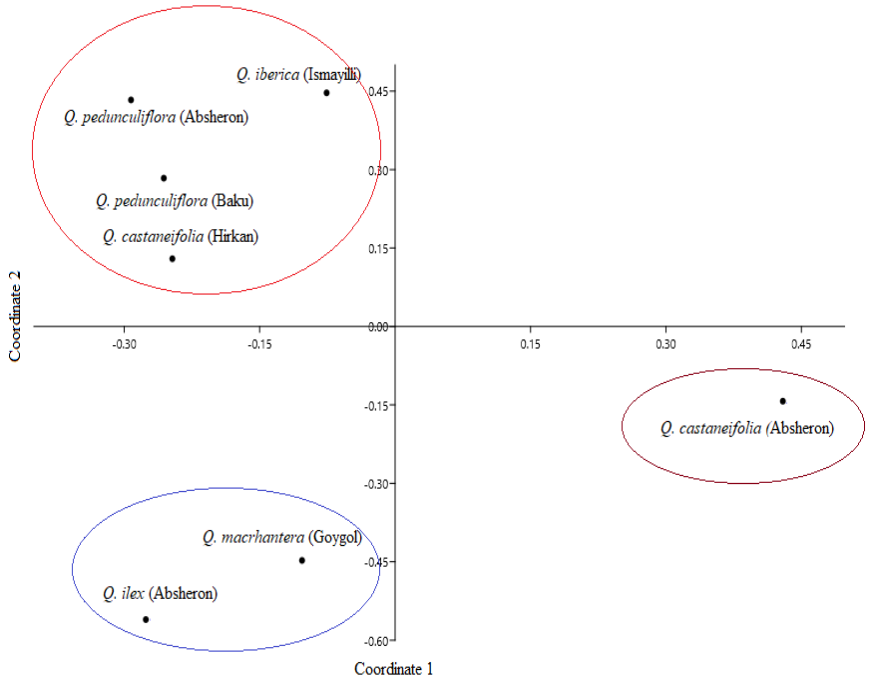

Fig. 4. Principal coordinate analysis (PCoA) showing Jaccard genetic similarity among studied oak species.

CONCLUSION

The present study reveals that the PCR-based fingerprinting technique by RAPD markers is informative for estimating the extent of genetic diversity as well as to determine the pattern of genetic relationships between different species of *Quercus*, with polymorphism levels sufficient to establish informative fingerprints with relatively fewer primer sets. We obtained high level of genetic diversity among Azerbaijan oak species from various regions. The information obtained from the present study could be of practical use for mapping the oak genome as well as for classical breeding. This study will help for future investigation aimed at defining the level of intra- and inter-specific genetic diversity and distinguishing hybrids among species. We hope the result of this study can provide an important input into determining efficient management strategies for conservation of valuable gene pool of *Quercus* in Caucasus.

REFERENCES

Abdessemed, S, Muzzalupo, I & Benbouza, H 2015, Assessment of genetic diversity among Algerian olive (*Olea europaea* L.) cultivars using SSR marker. *Scientia Horticulturae*, 192: 10-20.

Alikhani, L, Rahmani, MS, Shabanian, N, Badakhshan, H & Khadivi-Khub, A 2014, Genetic variability and structure of *Quercus brantii* assessed by ISSR, IRAP and SCoT markers. *Gene*, 552: 176-183.

Aliyeva, G 2021, Variations in leaf morphological and functional traits of *Quercus castaneifolia* C.A. Mey. (*Fagaceae*) in Azerbaijan. Russia, *Skvortsovia*, 7: 41-53; DOI: 10.51776/2309-6500_2021_7_2_41

Aliyeva, G, Mammadova, Z & Ojaghi, J 2021, Morphometric leaf variation in *Quercus* of Azerbaijan, *Bartın Orman Fakultesi Dergisi*, 23 (2), DOI: 10.24011/barofd.xxxx

Aliyeva, G, Mammadova, Z, & Ojagi, J 2020a, Evaluation of morphological traits and genotypes by multivariate statistical methods in some oak species. *Bulletin of Science and Practice*, 6: 10-18 https://doi.org/10.33619/2414-2948/59/01

Aliyeva, G, Mammadova, Z, Ojaghi J, Pourbabei, H 2020, b, Inter- and intra-population variations in leaf morphological and functional traits of *Quercus petraea* subsp. *iberica* under ecological factors in Azerbaijan, *Plant & Fungal Research*, 3: 61-68, http://plantfungalres.az/en/journals/49

Aminul, Islam, AKM, Anuar, N, Yaakob, Z & Osman, M 2016, Genetic diversity among candidate plus plants (CPPs) in jatropha (*Jatropha curcas*), *Pakistan Journal of Agricultural Sciences*, 53: 585-592.

Antic, M, Kajkut Zeljkovic, M & Duric, G 2020, Diversity assessment of wild cherry germplasm by using RAPD markers, *Bulgarian Journal of Agricultural Science* 26: 404-408.

Caspian J. Environ. Sci. Vol. 19 No. 3 pp. 457~468
DOI: 10.22124/CJES.2021.4932

Received: April 01. 2021 Accepted: June 22. 2021
Article type: Research

Ardi, M, Rahmani, F & Siami A 2012, Genetic variation among Iranian oaks (*Quercus* spp.) using random amplified polymorphic DNA (RAPD) markers. *African Journal of Biotechnology*, 11: 10291-10296

Arekhi, S, Heydari, M & Pourbabaei H 2010, Vegetation-environmental relationships and ecological species groups of the Ilam oak forest landscape, Iran. *Caspian Journal of Environmental Sciences*, 8: 115-125

Arghavani, A, Asghari, A, Shokrpour & M, Mohammaddost, C 2010, Genetic Diversity in Ecotypes of Two *Agropyron* Species using RAPD Markers, *Research Journal of Environmental Sciences*, 4: 50-56.

Arif, IA, Bakir, MA, Khan, HA, Al Farhan, AH, Al Homaidan, AA, Bahkali, AH, Al Sadoon, M & Shobrak M 2010, A Brief Review of Molecular Techniques to Assess Plant Diversity. *International Journal of Molecular Sciences*, 11: 2079-2096.

Ashley, MV, Backs, JR, Kindsvater, L & Abraham ST 2018, Genetic Variation and Structure in an Endemic Island Oak, *Quercus tomentella* and Mainland Canyon Oak, *Quercus chrysolepis*. *International Journal of Plant Sciences*, 179: 151-161, https://doi.org/10.1086/696023.

Attarod, P, Sadeghi, S, Pypker, TG & Bayramzadeh, V 2017, Oak trees decline; a sign of climate variability impacts in the west of Iran. *Caspian Journal of Environmental Sciences*, 15: 373-384

Aykut, Y, Emel, U & Tekin, BM 2013, Molecular diversity among Turkish oaks (*Quercus*) using random amplified polymorphic DNA (RAPD) analysis. *African Journal of Biotechnology* 12: 6358-6365.

Baig, NMR, Grewal, S & Dhillon S, 2008, Molecular characterization and genetic diversity analysis of citrus cultivars by RAPD markers. *Turkish Journal of Agriculture*, 33: 375-384.

Chen, S, Zhou, J, Chen, Q, Chang, Y, Du & Meng H 2013, Analysis of the genetic diversity of garlic (*Allium sativum* L.) germplasm by SRAP. *Biochemical Systematics and Ecology*, 50: 139-146. https://doi.org/10.1016/j.bse.2013.03.004

Degen, B, Yanbaev, R & Yanbaev Y 2019, Genetic differentiation of *Quercus robur* in the South-Ural. *Silvae Genetica*, 68: 111-115, DOI: https://doi.org/10.2478/sg-2019-0019.

Dnyaneshwar, W, Preeti C, Kalpana J & Bhushan, P 2006, Development and Application of RAPD-SCAR Marker for Identification of *Phyllanthus emblica* LINN, *Biological and Pharmaceutical Bullutein* 29: 2313-2316.

Dosmann, M & Aiello, A 2013, The Quest for the Hardy Southern Live Oak. *Arnoldia.* 70: 12-24.

Fernandes, JP, Costa, A, Rocha, CSJ & Santos C (2011). Genetic stability evaluation of *Quercus* suber L. somatic embryogenesis by RAPD analysis. *Pakistan Journal of Botany*, 43: 2727-31

Golan-Goldhirsh, A, Barazani, O, Wang, ZS, Khadka, DK, Saunders JA, Kostiukovsky V & Rowland, LJ 2004, Genetic relationships among Mediterranean *Pistacia* species evaluated by RAPD and AFLP markers. *Plant Systematics Evolution*, 246: 9-18.

Gürcan, K, Teber, S & Ercisli, S 2016, Genotyping by sequencing (GBS) in apricots and genetic diversity assessment with GBS-Derived Single-Nucleotide Polymorphisms (SNPs). *Biochemical Genetics.* 54: 854-885. https://doi.org/10.1007/s10528-016-9762-9.

Hamouda, M 2019, Molecular analysis of genetic diversity in population of *Silybum marianum* (L.) Gaertn in Egypt. *Journal of Genetic Engineering and Biotechnology*, 17: 12-25.

Jurksienė, G, Baranov, OY, Kagan, DI, Kovalevič-Razumova, OA & Baliuckas, V 2019, Genetic diversity and differentiation of pedunculate (*Quercus robur*) and sessile (*Q. petraea*) oaks. *Journal of Forestry Research*. DOI: 10.1007/s11676-019-01043-3.

Khadivi-Khub, A, Shabanian, N & Rahmani MS 2015, Genotypic analysis and population structure of Lebanon oak (*Quercus libani* G. Olivier) with molecular markers. *Tree Genetics & Genomes* 11: 102. https://doi.org/10.1007/s11295-015-0935-1.

Kumar J & Agrawal V 2019, Assessment of genetic diversity, population structure and sex identification in dioecious crop, *Trichosanthes dioica* employing ISSR, SCoT and SRAP markers. *Heliyon*, 5: e01346.

Kumar, M, Chaudhary, V, Sirohi U, Kumar Singh, M, Malik, S & Naresh RK 2018, Biochemical and molecular markers for characterization of chrysanthemum germplasm: A review. *Journal of Pharmacognosy and Phytochemistry*, 7: 2641-2652.

Caspian J. Environ. Sci. Vol. 19 No. 3 pp. 457~468
DOI: 10.22124/CJES.2021.4932

Received: April 01. 2021 Accepted: June 22. 2021
Article type: Research

Laborda, PR, Oliveira, KM, Garcia, AAF, Paterniani, M & De Souza, AP 2005, Tropical maize germplasm: what can we say about its genetic diversity in the light of molecular markers? *Theoretical and Applied Genetics* 111:1288-1299. DOI: 10.1007/s00122-005-0055-7.

Larsary, MK, Pourbabaei, H, Sanaei, A, Salehi, A, Yousefpour, R, & Ali, A 2021, Tree-size dimension inequality shapes aboveground carbon stock across temperate forest strata along environmental gradients *Forest Ecology and Management*: 496: 119482

Lopez-Aljorna, A, Bueno, MA, Aguinagalde, I & Martın, JP 2007, Fingerprinting and genetic variability in cork oak (*Quercus suber* L.) elite trees using ISSR and SSR markers. *Annals of Forest Science*, 64: 773-779.

Luo, J, Hou, BW, Niu, ZT, Liu W, Xue, QY & Ding, XY 2014, Comparative chloroplast genomes of photosynthetic orchids: insights into evolution of the *Orchidaceae* and development of molecular markers for phylogenetic applications. *PLoS ONE*, 9: e99016, https://doi.org/10.1371/journal.pone.0099016.

Luo, X, Li, HX, Wu, ZK, Yao, W, Zhao, P, Cao, D, Yu, HY, Li, KD, Poudel, K & Zhao, DG 2019, The pomegranate (*Punica granatum* L.) draft genome dissects genetic divergence between soft- and hard-seeded cultivars, *Plant Biotechnology Journal*: 1-14.

Menitsky, YL 2005, Oaks of Asia, Enfield: Science Publishers, USA, 549 p.

Miyashita, T, Kunitake, H, Yotsukura, N & Hoshino, Y 2015, Assessment of genetic relationships among cultivated and wild *Rubus* accessions using AFLP markers. *Scientia Horticulturae*, 193: 165-173.

Mohammed, IA, Gumaa, AN, Kamal, NM, Alnor, YS & Ali, AM 2012, Genetic diversity among some C*ucurbits* species determined by random amplified polymorphic DNA RAPD marker. *International Journal of Plant Research*, 2: 131-137. DOI:10.5923/j.plant.20120204.05

Naik, PK, Alam, MA, Singh, H, Goyal, V, Parida, S, Kalia, S & Mohapatra, T 2010, Assessment of genetic diversity through RAPD, ISSR and AFLP markers in *Podophyllum hexandrum*: a medicinal herb from the Northwestern Himalayan region. *Physiology and Molecular Biology of Plants.* 162: 135-48.

Neophytou, C, Aravanopoulos, FA, Fink, S & Dounavi A 2010, Detecting interspecific and geographic differentiation patterns in two interfertile oak species (*Quercus petraea* (Matt.) Liebl. and *Q. robur* L.) using small sets of microsatellite markers, *Forest Ecology and Management*, 259: 2026-2035.

Neophytou, C, Gärtner, SM & Vargas-Gaete, R 2015, Genetic variation of Central European oaks: shaped by evolutionary factors and human intervention? *Tree Genetics & Genomes*. 11: 79, https://doi.org/10.1007/s11295-015-0905-7.

Ojaghi, J & Akhundova E 2010, Genetic diversity in doubled haploids wheat based on morphological traits, gliadin protein patterns and RAPD markers. *African Journal of Agricultural Research*, 5: 1701-1712. https://doi.org/10.5897/AJAR09.754.

Ostadhashemi, R, Rostami, Shahraji, T, Mohammadi Limaei, S & Roehle, H 2014, Goal programming and analytical hierarchy process approaches for sustainable plantation. *Caspian Journal of Environmental Sciences*, 12: 233-244

Patel, HK, Fougat, RS, Kumar, S, Mistry, JG & Kumar, M 2015, Detection of genetic variation in *Ocimum* species using RAPD and ISSR markers. 3 *Biotechnology*, 5: 697-707.

Patel, P, Rajkumar, BK, Parmar, P, Shah, R & Krishnamurthy, R 2018, Assessment of genetic diversity in *Colletotrichum falcatum* Went accessions based on RAPD and ISSR markers. *Journal of Genetic Engineering and Biotechnology*, 16: 53-159.

Patzak, J, Paprštein, F, Henychová, A & Sedlák, J 2012, Comparison of genetic diversity structure analyses of SSR molecular marker data within apple (*Malus* × *domestica*) genetic resources. Genome 55: 647-665.

Ramanatha, Rao V & Hodgkin, T 2002, Genetic diversity and conservation and utilization of plant genetic resources. *Plant Cell, Tissue and Organ Culture*, 68: 1-19.

Ramanayake, S, Meemaduma, V & Weerawardene, T 2007, Genetic diversity and relationships between nine species of bamboo in Sri Lanka, using Random Amplified Polymorphic DNA. *Plant Systematics and Evolution*, 269: 55-61. https://doi.org/10.1007/s00606-007-0587-1

Randi, E & Lucchini, V 2002, Detecting rare introgression of domestic dog genes into wild wolf (*Canislupus*) populations by Bayesian admixture analyses of microsatellite variation, *Conservation Genetics* 3: 29-43.

Caspian J. Environ. Sci. Vol. 19 No. 3 pp. 457~468 Received: April 01. 2021 Accepted: June 22. 2021
DOI: 10.22124/CJES.2021.4932 Article type: Research

Rohlf, F 2000, NTSYS-PC numerical taxonomy and multivariate analysis system, Version 2.11L, Applied Biostatistics, New York

Saenz-Romero, C, Sniverly, A & Linding-Cisneros, R 2003, Conservation and restoration of pine forest genetic resources in Mexico, *Silvae Genetica*, 52: 233-237.

Salayeva, SJ, Ojaghi, JM, Pashayeva AN, Izzatullayeva VI, Akhundova EM, Akperov ZI 2016, Genetic diversity of *Vitis vinifera* L. in Azerbaijan. *Russian Journal of Genetics*, 52: 391-397, https://doi.org/10.1134/S1022795416030133.

Samaha, GM, Ahmed, MA & Abd El-Hameid, AR 2019, Assessment of growth and productivity of five peanut cultivars and genetic diversity using RAPD markers. *Bulletin of the National Research Centre*, 43: 168.

Schiller, G & Korol, L 2004, Genetic diversity assessment by random amplified polymorphic DNA of oaks in Israel. 2. *Quercus boissieri* Reut. *Israel Journal of Plant Sciences*, 52: 315-322.

Sen, S, Skaria, R & Muneer PM 2010, Genetic diversity analysis in piper species (*Piperaceae*) using RAPD markers. *Molecular Biotechnology*, 46: 72-79, DOI 10.1007/s12033-010-9281-6.

Shi, X, Wen, Q, Cao, M, Guo, X & Xu, L 2017, Genetic diversity and structure of natural *Quercus variabilis* population in China as revealed by microsatellites markers. *Forests*, 8: 495. DOI: 10.3390/f8120495.

Singh, V, Kudesia, R, & Bhadauria, S 2019, Assessment of genetic diversity in some Indian *Lablab purpureus*, L. bean genotypes based on RAPD Marker. Proceedings of the National Academy of Sciences, India Section B: Biological Sciences, DOI: 10.1007/s40011-019-01158-x.

Smith, JSC, Chin, EC, Shu, H, Smith, OS, Wall, SJ, Senior, ML, Mitchell, SE, Kresovich, S & Ziegle, J 1997, An evaluation of the utility of SSR loci as molecular markers in maize (*Zea mays* L.) comparisons with data from RFLPs and pedigree. *Theorotical Applied Genetics*, 95: 163-173.

Solomon, J, Shulkina, T & Schatz GE (Eds.) 2014, Red list of the endemic plants of the Caucasus: Armenia, Azerbaijan, Gerogia, Iran, Russia, and Turkey, St. Louis, Missouri Botanical Garden Press, 451 p.

Sorkheh, K, Masaeli, M, Chaleshtori, MH, Adugna, A & Ercisli, S 2016, AFLP-based analysis of genetic diversity, population structure, and relationships with agronomic traits in rice germplasm from north region of Iran and world core germplasm set. *Biochemical Genetica*, 54: 177-93, DOI: 10.1007/s10528-016-9711-7.

Subramanyam, K, Muralidhararao, D & Devanna, N 2009, Genetic diversity assessment of wild and cultivated varieties of *Jatropha curcas* (L.) in India by RAPD analysis, *African Journal of Biotechnology* 8: 1900-1910.

Talas, Oğraş, T, Koban, Baştanlar, E, Karakaş, Metin, Ö, Kandemir I & Özçelik, H 2017, Assessment of genetic diversity of rose genotypes using ISSR markers. *Turkish Journal of Botany*, 41: 347-355.

Thomas, KG & Bebeli, PJ 2010, Genetic diversity of Greek *Aegilops* species using different types of nuclear genome markers, *Molecular Phylogenetics and Evolution*, 56: 951-961.

Volis, S, Yakubov, B, Shulgina, E, Ward, D, Zur, V & Mendlinger, S 2001, Tests for adaptive RAPD variation in population genetic structure of wild barley, *Hordeum spontaneum* Koch. *Biological Journal of the Linnean Society*, 74: 289-303.

Yakovlev, IA & Kleinschmidt J 2002, Genetic Differentiation of Pedunculate Oak *Quercus robur* L. in the European Part of Russia Based on RAPD Markers. *Russian Journal of Genetics* 38: 148–155.

Yeasmin, L, Ali, MN, Gantait, S, & Chakraborty, S 2015, Bamboo: an overview on its genetic diversity and characterization. *3 Biotech*, 5: 1-11.

Zeinali, H, Tabaei-Aghdaei, SR, Arzani, A 2009, A study of morphological variations and their relationship with flower yield and yield components in *Rosa damascena*. *Journal of Agriculture Science and Technology*, 11: 439-448.

Bibliographic information of this paper for citing:

Aliyeva, G, Ojaghi, J, Rustamova, S 2021, Molecular diversity and phylogenetic analysis of Azerbaijan oaks (*Quercus* spp.) revealed by RAPD markers. Caspian Journal of Environmental Sciences, 19: 457-468

Caspian J. Environ. Sci. Vol. 19 No. 3 pp. 457~468
DOI: 10.22124/CJES.2021.4932
Received: April 01. 2021 Accepted: June 22. 2021
Article type: Research