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ABSTRACT

of the dissertation for the degree of Doctor of Philosophy

MORPHOLOGY AND GENETIC POLYMORPHISM OF SOME OAK (QUERCUS L.) TAXA IN THE AZERBAIJAN FLORA

Specialty: 2417.01 – Botany

Field of science: Biology

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GENERAL CHARACTERISTICS OF THE WORK

Relevance and degree of completion of the topic: There are many biologically rich and important areas on Earth, but unfortunately, these areas are seriously endangered. These areas are called biodiversity hotspots and cover 36 regions. The Caucasus region is one of the 25 hotspots of global biodiversity¹. In terms of the total number of taxa, the flora of Azerbaijan is much richer than other republics of the South Caucasus. The plant taxa found in the republic make up 66% of the total number of plant taxa growing in the Caucasus. There are 240 endemic plant taxa in our country. This is up to 6% of the total flora². On the basis of the order of the President of the Republic (3.10.2016, №2358) "National Strategy for the Protection and Sustainable Use of Biodiversity in the Republic of Azerbaijan for 2017-2020" was adopted. Monitoring, research, protection, efficient use of biological diversity using modern methods and improving the biodiversity information system were identified as key areas of activity. The Ministry of Natural Resources of the Republic of Azerbaijan and the institutes of the Azerbaijan National Academy of Sciences were entrusted with the implementation of the work³.

Soil erosion, landslides, regulation of soil and river water balance, global warming are also important global environmental problems. Forests, especially oak forests, play an invaluable role in solving these problems.

The purpose and tasks of the research: The purpose of the research work was to study morphological and genetic polymorphism

¹ Noss, R.F. How global biodiversity hotspots may go unrecognized: lessons from the North American Coastal Plain. Diversity and Distributions / Noss, R.F. Platt W.J., Sorrie B.A., // – 2015. 21(2), – p. 236-244.

² Tarkhnishvili, D. Historical biogeography of the Caucasus / D. Tarkhnishvili. – New York: Nova Science Publisher, – 2014. – 246 p.

³ Azərbaycan Respublikasında bioloji müxtətifliyin qorunmasına və davamlı istifadəsinə dair 2017-2020-ci illər üçün Milli Strategiya // Azərbaycan Respublikası Prezidentinin Sərəncamı. – Bakı, – 3 oktyabr, 2016. №2358.

in some oak taxa grown under different ecological conditions in the Azerbaijan flora. To achieve this goal, the following tasks were set:

- > collection of materials for morphological and genetic analyses from oak populations grown under different environmental conditions
- ➤ analysis of visually observed polymorphism in morphological traits by statistical methods
- > study of the dependence of morphological diversity on environmental factors
- > determination of identifying morphological factors for local oak taxa
 - > clarification of the status of oak taxa grown in Azerbaijan
- ➤ investigation of genetic diversity in oak taxa using molecular genetic markers
- > comparative analysis of results obtained from morphological and molecular-genetic studies
- ➤ determination of genetic distance, phylogenetic relationship, similarity and genetic polymorphism among different oak taxa.

Main points presented to the defense of the dissertation:

- The level of intra-population polymorphism is higher than inter-population polymorphism in oak taxa in different regions of Azerbaijan. The traits with the highest variation are leaf width/length ratio, shape factor, and leaf surface.
- Morphological polymorphism levels of *Q. robur* subsp. *Pedunculiflora* (K.Koch) Menitsky and *Q.ilex* L. taxa are higher compared to *Q. macranthera* subsp. *macranthera* (K.Koch) Menitsky and *Q. petraea* subsp. *iberica* (Stev.).
- RAPD primers are effective in assessing the genetic diversity of native oak taxa and studying phylogenetic relationships between oak populations and can be used as specific markers in the identification of local oak taxa.
- ullet Q.robur subsp. pedunculiflora and Q. petraea subsp. iberica taxa are phylogenetically closer to each other.

Scientific novelty of the research. For the first time in Azerbaijan, the results of morphometric measurements using CI-202

LESER AREA METER (USA) to assess intra-population and interpopulation morphological polymorphism in oak populations were evaluated by ANOVA- analysis of variance based on the RCBD method. Morphological traits were assessed using Principal Component Analysis (PCoA) and the best discriminating traits were identified for each oak taxa. Oak genotypes were grouped according to biomorphological characteristics by cluster analysis and the phylogenetic relationship between them was determined. For the first time in Azerbaijan, research was conducted on oaks at the molecular-genetic level using 10 RAPD markers. Molecular diversity of taxa, phylogenetic relationship between genotypes, genetic similarity between genotypes, etc. were studied. The obtained results were analyzed using modern statistical methods and important statistical parameters such as PIC (polymerphism information content), EMR (effective multiplex ratio), and MI (marker index) were evaluated.

Theoretical and practical significance of the research: The dissertation presents the latest literature data from local and foreign sources about the ecology of the oak plant: information on the need for soil, water, light, necessary measures for the establishment of new oak forests. This theoretical knowledge can be used to establish new oak forests in Azerbaijan following the requirements of the geographical area, to restore biodiversity in Karabakh, as well as to green parks and alleys in Baku. The results of molecular studies can be used in the future as a comparative study of intraspecific and interspecific diversity of oaks in the country's flora, in the identification of hybrids between taxa, mapping local oak genomes, as well as preliminary research in classical selection work.

During the research, new results important for the morphological, taxonomic, and genetic characteristics of oaks were obtained and presented in the dissertation. This information can be used as an auxiliary in the creation of the "Azerbaijan Plant Definer" and the 3rd edition of the "Red Book" of the Republic of Azerbaijan on plants, the new edition of "Azerbaijan Flora", as well as various fundamental scientific works on the flora of Azerbaijan. Master's and doctoral students who will conduct research on the biodiversity of the Azerbaijani flora and oaks

can use the dissertation as literature material.

Approbation and application of the research: The main points of the work were presented at local and international conferences: 3rd International Symposium on Eurasian Biodiversity (SEAB, Minsk, Belarus, July 05-08, 2017), International scientific conference on "Impact of climate change on plant biodiversity" (Baku, 2017), II International Scientific Conference of Young Researchers, (Baku, 2018), Scientific conference "New Challenges in Botanical Research" dedicated to the 90th anniversary of Academician Vahid Jalal oglu Hajiyev (Baku, 2018), 4th International Symposium on Eurasian Biodiversity (SEAB, Kyiv, Ukraine, 03-06 July 2018), a conference of young scientists and researchers on "Innovations and global challenges in modern biology and agricultural sciences", dedicated to the 90th anniversary of academician J.A.Aliyev (Baku, 2018), Scientific conference of young scientists and specialists on "Innovation and traditions in modern botany" dedicated to the 130th anniversary of Academician Alexander Grossheim (Baku, 2019), Winter School of Young Biologists organized by ANAS, Division of Biological and Medical Sciences and the Council of Young Scientists and Specialists (Baku, 2019), Second International Scientific Conference of Young Scientists and Specialists on multidisciplinary approaches to solving modern problems of fundamental and applied sciences (Baku, 2020), dedicated to the 75th anniversary of the Azerbaijan National Academy of Sciences, Scientific-Practical Conference of students, masters and doctoral students (dissertators) on "Environmental problems and strategies for its preservation: vision for the future" dedicated to the "Science Day" organized by the Council of Young Scientists and Specialists of the Institute of Soil Science and Agrochemistry (Baku, 2020), IV International Scientific Conference of Young Researchers dedicated to the 97th anniversary of National Leader Heydar Aliyev (Baku, 2020), Ponto-Caspian and Caucasus Region: changes in the conditions of integration and isolation of ecosystems, phylogenesis, geology, ecology and geography of living things, Multidisciplinary International Conference (Baku, 2020), Scientific seminar on "Molecular analysis of genetic diversity of some oak taxa (Quercus spp.) in Azerbaijan" held in the online format (Baku, 2021), whithin the

framework of the events dedicated to "Science Day", X International Scientific Conference of Young Scientists and Researchers on "Scientific Achievements and Challenges in Biology" (Baku, 2021), Conference on "Biodiversity of Karabakh, land and water resources: past, present and future" (Baku, 2021), International Congress of Health Sciences and Innovations (Baku, 2021), Karabakh, II International Congress of Applied Sciences (Baku, 2021).

28 scientific works containing the main provisions of the dissertation were published, including 15 articles (4 abroad) and 13 theses (3 abroad). The articles were published in journals indexed in reputable databases. Thus, two articles were published in journals included in Web of Science and Scopus, one in Agris, and one in РИНЦ.

The organization where the dissertation was performed: The research was carried out at the Institute of Dendrology and Institute of Molecular Biology and Biotechnologies of the Azerbaijan National Academy of Sciences.

Separate volumes of structural units of the dissertation: The dissertation consists of an introduction, 7 chapters, conclusions, recommendations, a list of literature and a list of abbreviations used in the dissertation. 236 resources were used in the research, of which 45 are in Azerbaijani, 12 in Turkish, 12 in Russian, and 167 in English. The dissertation consists of 157 pages, including 24 figures and 33 tables.

MAIN CONTENT OF THE DISSERTATION Chapter I. Study and prospect of oaks

Chapter I of the dissertation is a review compiled on the basis of local and foreign literature. The evolution and classification of oaks, the history and current state of the study of oaks, taxa diversity, distribution in the world and in Azerbaijan, including Karabakh⁴, the

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⁴ Aliyeva, G.N. Research on high genetic resources of oak forests in Karabakh and highlighting benefits of reconstruction of oak forests to our ecology and economic development // Journal of Life Sciences and Biomedicine, − 2021. 3 (76) №2, − p. 107-114. http://dx.doi.org/10.29228/jlsb.29

role of oaks in the formation of biodiversity, the symbolic, ecological and economic values of oaks for our country, ethnobotanical application, etc. are included in the chapter.

Chapter II. Materials and methods of the research.

The objects of research are some oak taxa growing under different ecological conditions in Azerbaijan (taksonları (*Q. petraea* subsp. *iberica* (Stev.), *Q. macranthera* subsp. *macranthera* (K.Koch) Menitsky, *Quercus castaneifolia* C.A.Mey., *Q. robur* subsp. *pedunculiflora* (K.Koch) Menitsky, *Q.ilex* L.) and the subject is the study of morphological and genetic polymorphism in these taxa.

To study the morphological and genetic polymorphism of known oak taxa, leaf samples were collected from different regions of Azerbaijan in June-August 2017⁵. In general, 1160 leaf samples were taken from 14 populations: Pedunculate oak from Ganja, Baku (Central Botanical Garden) and Absheron (Mardakan Arboretum), Oriental oak from the Goygol region (Goygol National Park), Chestnut leaf oak from Lankaran (Lankaran plain, Hirkan National Park), from Baku (Botanical Garden) and Absheron (Mardakan Arboretum), Georgian oak from Ismayilli, Gabala and Baku (Botanical Garden), and Holm Oak from Baku (Central Botanical Garden and Officers Park) and Absheron (Mardakan Arboretum).

Six morphometric parameters (LL-leaf length, LW-leaf width, LSA-leaf surface area, LP-leaf perimeter, R- Ratio (R = LL/LW) and F-factor (leaf shape coefficient) (LW/LP) were measured using CI-202 LESER AREA METER (USA)⁶.

The width, length, and mass of the acorns were measured to study intra-population variation in oak acorns⁷. The mass of the acorns was measured on an electronic scale (EK-610i) with an accuracy of 0.01 g,

⁶ Jensen, J.R. Detecting shape variation in oak leaf morphology: a comparison of rotational-fht methods // American Journal of Botany, – 1990. 77(10), – p. 1279-1293.

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⁵ Bruschi, P. Morphological and molecular diversity among Italian populations of *Quecus petraea* (Fagaceae) / P. Bruschi, G. Vendramin, F. Bussotti, [et al.] // Annals of Botany, – 2003. 91, – p. 707-716.

⁷ Bakiş, Y. Morphometrical Analysis of Oaks (*Quercus* L.) Acorns in Turkey: / Ph.D. Thesis. / – Bolu, Baysal University, 2005. – 72 s.

and the width (widest part) and the length (from the base to the tip of the acorn) were measured using a High Contrast Vernier Caliper (0.1 cm).

Variations in some physiological indicators of the leaf of oak taxa grown under different ecological conditions were studied under the influence of different environmental factors and the correlation of these changes with morphological polymorphism was explored⁸.

Ten RAPD markers were used to study genetic polymorphism. For this purpose, DNA was isolated⁹ from oak leaves by the CTAB method, the purity degree of the separated DNA was examined using the spectrophotometric method, PCR was applied with 10 RAPD primers, electrophoresis of PCR products was performed on 1.5% agarose gel¹⁰, UVITEK Gel Documentation System was used to obtain the gel image. The obtained results were statistically analyzed.

Compilation and mathematical analysis of the sequence of variations were conducted according to the morphological features of leaves and acorns. For the centralized preparation of numbers for further analysis, the Excel computer program was used. Detection of the degree of variability in traits was performed using analysis of variance (ANOVA) and Principal Component Analysis (PCA) based on RCBD (Randomized Complete Block Design). Two statistical tests, KMO (Kaiser-Meyer-Olkin) and Bartlett tests were used to verify the statistically correct performance of the PCA. Statistical analysis was performed using SPSS 16, PAST, and MSTATC statistical computer programs. To evaluate the effectiveness of RAPD primers for genetic analysis of oak samples, statistical parameters such as PIC, EMR, MI, resolving power (RP) were determined. Cluster analysis of oak was performed and a dendrogram was constructed using SPSS program and UPGMA (unweighted pair group with

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⁸ Bussotti, F. Structural and functional traits of *Quercus ilex* in response to water availability / F. Bussotti, B. Davide, G. Paolo [et al.] // Environmental and Experimental Botany, – 2002. 47, – p. 11-23.

⁹ Murray, M.G. Rapid isolation of high molecular weight plant DNA. / M.G.Murray, W.F Thompson // Nucleic Acids Research, – 1980. 8, – p. 4321-4325.

Ardi, M. Genetic variation among Iranian oaks (*Quercus* spp.) using random amplified polymorphic DNA (RAPD) markers / M. Ardi, F. Rahmani, A. Siami // African Journal of Biotechnology, – 2012. 11(45), – p. 10291-10296.

arithmetic average) based on Jaccard similarity coefficient according to RAPD markers. Based on cluster analysis, genotypes were divided into groups taking into account all morphological features. The genetic relationship between oak taxa was also studied using Principal Coordinate Analysis (PCoA). PCoA was performed using RAPD markers based on the Jaccard similarity coefficient.

Chapter III. Bioecological characteristics and ecological groups of oaks

3.1.Chestnut-leaved oak

At relatively low altitudes, in the Hirkan flora, the dominance of chestnut-leaved oak (*Q.castaneifolia*) and hornbeam (*Carpinus*) is observed in the forest and hornbeam oak type forest is formed. Besides, various taxa of walnut (*Pterocarya* Kunth), alder (*Alnussubcordata* C.A.Mey) and elm (*Ulmus* L.) are also found in the areas. In the lower layers, dryopteris, viola, primula, and urtica can be found. In the upper mountain belt, chestnut-leaved oak grows together with ironwood, Georgian oak, and oriental oak.

3.2. Oriental oak

On the slopes of Kepaz mountain, in the territory of Goygol National Park, eastern oaks and hornbeam trees grow, and the border of the forests reaches 2400 m. At this height, oak groves consisting of middle-aged oaks formed by shoots are noticeable. The height of the trees is 6-8 m, the density reaches 8-12 cm. In the lower levels, willow, euonymus, rose hip, cotoneaster, and honeysuckle occur. Juniper trees are observed rare, and as the forest thins, the number of juniper trees increases. In some places, an ecosystem consisting of beech and oak is formed, which are alternated with rose hip and spiraea.

3.3. Georgian oak

One of the populations from which samples of Georgian oak were collected is in the forest ecosystem located in the Kichik Pirali village, Gabala region. Georgian oak forms a mixed forest type together with walnut, hornbeam, tilia, alder, willow and chestnut. In the second layer of the forest, there are shrubs such as medlar,

hawthorn, blackberry, and rose hip. In the lower layer, grass plants such as clover, mentha, plantago, and thyme are encountered. Another population is located in the Basgallar settlement, Ismayilli district, at an altitude of 1081 m above sea level, it is an area consisting mainly of hills. The main edificator of the forest ecosystem formed here is the Georgian oak. Along with the Georgian oak, elm and hornbeam are also encountered. Plants such as blackberry, hawthorn, rose hip, honeysuckle, berberis, crab apple, beech, etc. form the lower layer.

3.4. Pedunculate oak

In the Ganja-Gazakh Plain, located between the Kura River and the Lesser Caucasus, at an altitude of 400 m above sea level, the ecosystem consisting of pedunculate oak protects crops from strong winds and regulates the water balance of rivers and soil. Pedunculate oaks with a height of 20-25 m and a diameter of 60-80 cm together with elm form the first layer. In addition to the pedunculate oak, there are hornbeam, alder, tilia, ash and pistachio trees in the ecosystem.

3.5. Holm oak

It grows naturally in the west and east of the Mediterranean Sea. It was introduced to Azerbaijan (the Central Botanical Garden) in the 1980s by H. Aliyev. The holm oak is an evergreen tree with a large trunk and broad canopy, 25 m tall and 60 cm in diameter. Since the holm oak taxa is tolerant to frost, heat, shade and drought, not demanding on the soil, and grows quickly, it is desirable to transfer it to the natural flora in Azerbaijan, as well as in Karabakh.

The chapter also contains information about the biology of oak taxa, oak protection, natural recovery and failure, and measures taken to protect oaks in the world and in our republic.

Chapter IV. Morphological polymorphism

In chapter IV of the dissertation, morphological polymorphism of leaves is reflected, morphological features for each research object are determined, according to the obtained results, the names of local oak taxa were specified according to Menitsky¹¹ and international nomenclature¹² (The World Flora Online –WFO).

4.1. Interspecific polymorphism of morphological features in leaves. The ability of individual genotypes to generate different phenotypes in response to changes in the environment, or phenotypic plasticity is the basis of population sustainability and is a key element in taxa evolution and ecological interactions, ¹³. Oaks show a high level of variation because they form a wide geographical area ¹⁴. Oak trees have characteristics such as high levels of phenotypic plasticity, interspecific gene flow, and genetic variation, which have significantly contributed to the genesis of hundreds of taxa, subtaxa, and ecotypes.

During the analysis of morphometric results based on the RCDB method, the values of CV and LCD (Table 1) showed high reliability of the analysis and allowed performing other statistical analyses.

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Leaf characters	Repeat	Genotype	Error	LSD %5	C.V %				
df	1	90	90	-	-				
Leaf area (cm ²)	4.98*	352.88**	1.63	4.55	10.38				
Leaf length (cm)	0.06 ^{n.s}	2.586**	0.805	2.16	9.28				
Leaf width (cm)	0.049 ^{n.s}	1.587**	0.758	1.28	5.98				
Leaf perimeter (cm)	2.65**	3.25**	0.725	2.89	4.96				
Ratio	0.182*	189.2**	1.28	4.89	3.89				
Factor	0.497**	98.89**	2.09	1.89	11.28				

^{**}It shows significant at the 0.01 level (2-tailed).* It indicates significant at the 0.05 level (2-tailed).

 11 Menitsky, Y.L. Oaks of Asia / Y.L.Menitsky. – USA: Science Publishers of Enfield Press, – 2005. – $549\ p.$

Wund, M.A. Assessing the impacts of phenotypic plasticity on evolution // Integrative and Comparative Biology, -2012. 52, -p. 5-15.

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¹² The World Flora Online [Electronic resource] / – 2017. URL: http://www.worldfloraonline.org

¹⁴ Aldrich, P.R. *Quercus*. Wild Crop Relatives: Genomic and Breeding Resources, Forest Trees / P.R. Aldrich, J. Cavender - Berlin Heidelberg: Bares Springer-Verlag, – 2011. – 187 p. 89-131

Based on the ANOVA of morphological polymorphism caused by various environmental factors in different oak taxa from various regions of Azerbaijan, the level of intra-population polymorphism was higher than that of inter-population polymorphism. The highest variations were found for the traits: R (LL/LW) (CV=110.03%), F (CV=53.12%), and LSA (CV=34.01%) (Table 2) 15 .

4.2. Evaluation of traits and genotypes using multi- dimensional statistical methods. Two statistical tests, KMO and Bartlett tests were used in performing statistically correct Principal Component Analysis.

Statistical parameters on the studied traits

Table 2

Statistical parameters on the statica traits							
	Area	Length	Width	Perimeter	Ratio	Factor	
Min	23.96	13.68	4.54	95.87	1.576	0.01	
Max	125.39	23.84	16.75	187.78	27.01	0.12	
Average rate	57.62	19.63	7.81	140.69	3.19	0.042	
Standard error	±2.05	±0.22	±0.17	±2.15	±0.37	±0.01	
Variation	384.05	4.48	2.71	421.35	12.33	0.01	
Standard discriminant	19.59	2.12	1.65	20.53	3.51	0.02	
Median	56.19	19.71	7.54	144.97	2.68	0.04	
CV	34.01	10.78	21.06	14.59	110.03	53.12	

As a result of the Principal Component Analysis, 3 indicator elements explained 86.97% of the whole variations between genotypes¹⁶ (Table 3). As a result, all analyses were performed based on 3 selected indicator elements (PCA1, PCA2, and PCA3). Table 3 shows the values of the indicator elements obtained on the basis of traits. Through the values of these elements, it is possible to select effective genotypes based on one or more traits.

Aliyeva, G. Evaluation of Morphological Traits and Genotypes by Multivariate Statistical Methods in Some Oak Species / G. Aliyeva, Z. Mammadova, J. Ojagi // Bulletin of Science and Practice, − 2020. 6 №10, − p.10-18.

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Aliyeva, G. Variation analysis of leaf morphological traits in some oak species (*Quercus* sp.) Azerbaijan Republic / G.Aliyeva, Z.Mammadova, J.Ojaghi // Advances in Biology & Earth Sciences, – 2020. 5, No.3, – p. 232-240.

Using PCA, the best discrimination traits were established for the genotypes: for the taxa *Q. robur* subsp. *pedunculiflora*, *Q. petraea* subsp. *iberica* and *Q. macranthera* subsp. *macranthera* - LL and R, for *Q. castaneifolia* - LSA, LW, and F, and for *Q.ilex* L. - R¹⁷.

 $\label{thm:component} Table \, 3$ The results of the component analysis for each studied trait

Morphological characters	PCA1	PCA2	PCA3
Leaf area	0.13	0.60	0.27
Leaf length	0.29	-0.05	0.54
Leaf width	-0.01	0.50	0.35
Perimeter	0.09	-0.201	0.57
Ratio	0.94	-0.01	-0.27
Factor	-0.06	0.58	-0.33
Variation percentage	39.29	32.49	15.19
Total variation	39.29	71.78	86.97

4.3. Determination of interspecific genetic distance based on morphological traits. The studied oak genotypes were divided into 4 groups in a dendrogram based on a cluster analysis of biomorphological traits (Figure 1).

According to the obtained results, morphological distances of the taxa *Q. macranthera* subsp. *macranthera*, *Q.robur* subsp. *pedunculiflora*, *Q. petraea* subsp. *iberica* spread in Azerbaijan are closer, these taxa belong to the subgenus *Quercus* of the genus *Quercus*. The obtained result coincides with foreign literature data¹⁸.

4.4. Intrataxa, intra-population, and inter-population polymorphisms of morphological traits in oak leaves.

Inter-population statistical analysis of Georgian oak revealed the

¹⁷ Aliyeva, G. Morphometric leaf variation in *Quercus* of Azerbaijan. / G. Aliyeva, Z. Mammadova, J. Ojaghi // Journal of Bartin Faculty of Forestry, – 2021. 23(2), – p. 579-585.

Denk, T. The oaks of western Eurasia: Traditional classifications and evidence from two nuclear markers / T.Denk, G.Grimm // TAXON, – 2010. 59 (2), – p 351–366.

highest variations in morphological traits, such as LSA and F (CV=34.6% and 32.23%, respectively). The weakest variation was observed in the R parameter with a value of 11.03%. For other traits (LL, LW, LP, R) the distribution was normal (CV= 11-20%).

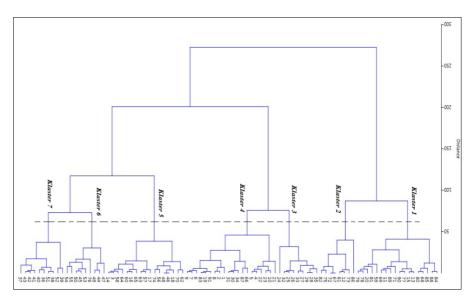


Figure 1. Grouping of some oak samples of Azerbaijan according to biomorphological-quantitative characteristics based on Euclidean distance

The leaves collected from the Ismayilli population differ from other populations by the high values of LSA, LL, and R. Leaf samples collected from the Baku population showed high values for the traits LW and LP. The highest index of leaf shape coefficient (F) was recorded in the Gabala population. Georgian oak leaves collected from the Gabala population are relatively small compared to other populations¹⁹.

Based on the statistical analysis of the morphometric results, the

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Aliyeva, G. Inter- and intrapopulation variations in leaf morphological and functional traits of *Quercus petraea* subsp. *iberica* under ecological factors in Azerbaijan / G. Aliyeva, Z. Mammadova, J. Ojaghi // Plant & Fungal Research – Baku: – 2020. 3(2), – p. 61-68.

taxonomic status of the Georgian oak taxon in the local flora was clarified using Menitsky's determinant and the WFO system: *Quercus petraea* subsp. *iberica*.

The study of inter-population diversity in chestnut-leaved oak trees based on the results of analysis of variance (ANOVA) showed that LSA in oak leaves from Hirkan National Park (HNP) (53.88 \pm 2.53 cm²) was smaller compared to this parameter in leaves of oak from Lankaran plain (LP) (65.68±2.07 cm²) and Mardakan arboretum (MA) (66.26 \pm 2.18 sm²) populations. ANOVA showed a statistically significant difference of 5% for LSA (LSA = 3.99%). The longest leaves were collected from the LP population (19.23 \pm 0.91 cm), the leaves of other populations were relatively short (HNP $(16.30 \pm 0.81 \text{ cm})$ and MA $(18.98 \pm 0.92 \text{ cm})$). There is a slight difference in the LP trait for the populations of HNP (103.98 \pm 2.11 cm) and LP (115.94 \pm 2.54 cm). However, an increase in this parameter (139.5 \pm 2.73 cm) was observed in the MA population, the trait distribution was moderate according to this parameter, CV = 10.16%. The lowest value for the ratio (LL / LSA) (2.63 \pm 0.36) was observed in the MA population. There is a slight difference in the value of R between the populations of HNP (4.82 \pm 0.29) and LP (5.01 \pm 0.26). The highest value of the leaf shape coefficient (0.08 ± 0.001) was observed in HNP. Although the lowest value for this parameter was observed in the MA population (0.04 \pm 0.001) (CV = 45.43%), the distribution was normal²⁰.

The highest variation of the studied morphological parameters of the leaf, such as LSA, LP, and LW was observed in oriental oak. The value of the coefficient of variation on these traits was equal to 44.32%, 38.16%, and 25.42%, respectively, the distribution was average. The maximum, minimum, average, and median values of all studied traits were analyzed. The weakest variation was found for LW

Aliyeva, G.N. Variations in leaf morphological and functional traits of *Quercus castaneifolia* C.A Mey. (*Fagaceae*) in Azerbaijan // Skvortsovia, – 2021, 7(2): – p. 41–53. DOI:10.51776/2309-6500_2021-7_2_41

(15.35%) and R (17.65%), the distribution was normal²¹.

Based on the statistical analysis of the morphometric results, the taxonomic status of the eastern oak taxon was specified in the local flora using Menitsky's determinant and the WFO system: *Quercus macranthera* subsp. *macranthera* (K.Koch) Menitsky.

Inter-population statistics of morphological traits were studied in pedunculate oak. A high coefficient of variation in traits indicated a high level of polymorphism, and the distribution of traits in populations was moderate (LSA, R, and F) and normal (LL, LW, and LP). According to the inter-population variations, there was no significant difference in the morphological traits of the leaves in pedunculate oaks grown in the Central Botanical Garden and Dendropark research base. However, the leaves of pedunculate oak trees grown in the natural flora of Ganja were relatively small compared to the other two populations. Thus, the highest values of LSA, LW, and LP were recorded in the Central Botanical Garden population, and the highest values of LL and R were recorded in the Mardakan Arboretum population. According to the leaf shape coefficient, no difference was observed in the populations.

Based on the statistical analysis of the morphometric results, the taxonomic status of the pedunculate oak taxon was determined in the local flora using Menitsky's determinant and the WFO system: *Quercus robur* subsp. *pedunculiflora* (K.Koch.) Menitsky.

In holm oak, the highest inter-population variation of traits was recorded for LSA (45.27%) and the leaf shape coefficient (38.63%). The weakest variations were observed for R (12.60%) and LW (15.60%) traits. The analysis of separate populations showed that the leaves of holm oak grown in the Mardakan arboretum were larger than in other populations, as the highest values for all morphological traits studied, except F, were recorded in this population. In the Central Botanical Garden, the olm oak leaves were smaller. In this population, the lowest values were observed for LSA, LP, LL, and LW traits.

²¹ Aliyeva, G. Variability of morphological characteristics of Caucasian oak (*Quercus macranthera* subsp. *macranthera* Fisch. & Mey.ex Hohen.) acorns // Elmi Xəbərlər, Gəncə − 2021, №3(36), − s. 120-125.

Although the holm oak leaves in the Officers' Garden were of medium size, the minimum values of the F and R traits were observed in this population ²².

Chapter V. Variation of morphological parameters of acorns

Within the population, distribution of the traits such as acorn length and acorn width was found to be medium in the taxa Q.robur subsp. pedunculiflora (13.9 %), Q. petraea subsp. iberica (14.2 %), Quercus castaneifolia (12.9 %) and Q.ilex (19.6 %) and normal in Q. macranthera subsp. macranthera (32.4%). The distribution of the acorn width trait in Quercus castaneifolia (26.84%) was normal. It was asymmetrical in *Q.ilex* (51.16%), while in other taxa, it was moderate. The distribution of the acorn mass trait in *Q.ilex* was assymmetrical (66.3%), and in other taxa, it was normal (CV>25%). No weak distribution of any morphometric parameters of the acorn was observed in the population. The fact that the distribution in the population is moderate, normal, and asymmetrical in terms of traits indicates a high morphological polymorphism of acorn in the population. This indicates the high adaptability of the taxa and is very important for the conservation of the taxa.

Chapter VI. Correlation of some physiological parameters of leaves with morphological polymorphism

The effect of environmental factors on the physiological parameters of leaves in oak taxa and the correlation of the studied physiological parameters with the morphometric parameters of the leaves have not been studied in Azerbaijan. In the research, the variation of some physiological parameters of the leaves of 5 oak taxa grown under different ecological conditions (Table 5.) was compared.

²² Məmmədova, Z.Ə. Daş palıdda (*Q.ilex* L.) yarpaqların variasiaya sırasına əsasən morfoloji polimorfizmin analizi / Məmmədova, Z.Ə., Əliyeva, G.N. // Bakı: AMEA-nın Xəbərləri (Biologiya və Tibb elmləri), – 2017. cild 72, №2, – s. 154-158.

Table 5 Correlation of some physiological parameters with morphological polymorphism in leaves.

	porymor phism in reaves:								
Ph	ysiological								
	parameters	LMA	WC	RWC	J	LA			
Tax	a,	(mg cm2)	(%)	(%)	(mg cm ²)	(cm2)			
popu	ılations								
1	HNP	7.42±0.61	58.03±1.62	71.39±1.82	7.79 ± 0.56	55.66±2.53			
	LP	6.24±0.52	57.73±1.58	73.34±1.95	8.83±0.66	64.68±2.07			
	MA	3.77±0.11	40.32±1.05	70.39±1.82	5.85±0.38	69.1±8.12			
2	NB	1.46±0.10	8.41±1.15	37.46±1.82	0.13±0.01	42.23±2.53			
	İsmayıllı	6.81±0.91	54.26±1.65	84.15±2.03	8.08±1.01	92.56±2.07			
	Qabala	7.16±1.14	62.23±1.82	83.38±2.13	8.24±1.08	67.26±2.18			
3	Ganja	6.43±0.76	50.50±4.21	72.98±5.22	6.54±0.12	76.18±5.78			
	NB	10.12±1.03	19.44±3.98	53.38±3.12	2.44±0.52	57.30±3.65			
	MD	12.48±1.14	16.43±6.23	45.75±6.19	2.45±0.60	48.87±4.04			
4	MA	8.23±0.97	30.27±2.17	48.27±2.92	3.67±0.09	41.05±3.23			
	OP	10.94±1.21	33.33±2.85	55.34±2.83	5.47±1.11	29.26±2.12			
	CBG	6.49±0.94	41.80±1.93	68.86±2.73	4.54±1.23	30.84±1.32			
5	GNP	6.44±1.15	52.38±3.49	72.54±3.22	7.09±1.37	78.1±9.79			

1-chestnut-leaved oak, 2- georgian oak, 3- pdunculate oak, 4- holm oak, 5- oriental oak, LMA-Leaf Mass per Area, WC-Water Content, RWC-Relative Water Content, J-juiciness, LA-leaf area, HNP-Hirkan National Park, LP- Lankaran Plain, MA-Mardakan Arboretum, CBG-Central Botanical Garden, Officers Park -OP, GNP-Goygol National Park

Physiological parameters of leaves in oak taxa (LMA-Leaf mass per area, WC-water content, RWC-relative water content, J-juiciness) are positively correlated with the average annual precipitation and altitude but negatively correlated with the average annual temperature. Changes in leaf sizes, especially, leaf surface area, have a positive correlation with changes in these indicators.

Chapter VII. Genetic polymorphism

7.1. Level of polymorphism and marker effectiveness. For the first time in Azerbaijan, scientific research has been conducted on oaks at the genetic level. Thus, during the RAPD-PCR analysis, a total of 113 bands were synthesized, 91 of which were polymorphic on 10

primers (Table 6). The main distribution area of the fragments was in the range of 250-3500 bp. The highest number of polymorphic bands was observed with OPB-02, and the lowest number of polymorphic bands was observed with OPB-05 primer. The average number of polymorphic bands was 9.1. The highest polymorphism (92.31%) was recorded with the primer OPD-05, and the weakest polymorphism was recorded with the primer OPB-05 (57.14%).

Table 6
Indicators of polymorphism and genetic diversity determined with RAPD primers in oak genotypes

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	CATCCCCTG	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

The highest values of polymorphic information content-PIC, which is considered the most important statistical parameter, were observed with primers OPD-05 (0.96), OPB-03 (0.95), and OPB-01 (0.94). The weakest values of PIC were observed with OPB-05 (0.48), and the average value for each primer was 0.75²³.

7.2. Markers, specific to taxa. Some amplified RAPD bands are only observed in certain taxa. Therefore, such RAPD primers can

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²³ Əliyeva, G.N., Azərbaycanin bəzi palıd (*Quercus* spp.) növlərinin genetik müxtəlifliyinin molekulyar analizi / G.N.Əliyeva, C.M.Ocaqi, S.M.Rüstəmova, [və b.] // AMEA-nın Məruzələr jurnalı, -Bakı: −2021. LXXVII, №1-2, −s. 33

be used as a specific marker for the identification of these taxa. Among the amplification products of the 10 primers used in the study, 22 RAPD bands had an identifying nucleotide sequence for the taxa under study. However, in the current study with OPB-04 and OPD-08 primers, specific bands were not identified for any taxa. A maximum of five unique bands were found in 3 oak taxa by OPD-11 primer. Detailed information on the specific bands for the identified oak taxa is given in Table 7.

Table 7
Specific RAPD markers for each studied oak taxa

Primer	Fragment	Size	Taxa
Filliei	No.	(bp)	1 axa
OPA-03	4	1200	Q.macranthera subsp. macranthera
	6	650	Q. macranthera subsp. macranthera
OPA-04	3	950	Q. castaneifolia
	5	750	Q. castaneifolia
	7	500	Q. petraea subsp. iberica
OBP-01	2	2500	Q.macranthera subsp. macranthera
	4	700	Q.robur subsp. pedunculiflora
	8	600	Q. robur subsp. pedunculiflora
	10	350	Q. ilex
OPB-02	14	450	Q. petraea subsp. iberica
OPB-03	2	1500	Q. castaneifolia
	4	950	Q.macranthera subsp. macranthera
OPB-05	7	450	Q. ilex
OPD-05	3	2500	Q. petraea subsp. iberica
	5	1500	Q. castaneifolia
	10	550	Q. petraea subsp. iberica
	12	450	Q.macranthera subsp. macranthera
OPD-11	3	2000	Q.robur subsp. pedunculiflora
	7	950	Q. petraea subsp. iberica
	9	750	Q. macranthera subsp. macranthera
	13	450	Q. petraea subsp. iberica
	15	350	Q.robur subsp. pedunculiflora

7.3. Similarity matrices based on RAPD markers. In RAPD-PCR analysis, binary matrices synthesized using amplified fragments of oak taxa were used to calculate the Jaccard similarity coefficient for the comparison of genotypes (Table 8). The approximate similarity fluctuates between 0.333 and 0.818. Based on the results of the study, genotypes *Q. robur subsp. pedunculiflora* (Baku) and *Q. robur* subsp. pedunculiflora (Absheron) are closely related to each other and have the highest genetic similarity (0.818). The lowest genetic identification value (0.333) was registered in the genotypes *Q. ilex* (Absheron) and *Q. castaneifolia* (Absheron), followed by *Q. robur* subsp. pedunculiflora (Baku) *Q. castaneifolia* (Absheron), *Q. robur* subsp. pedunculiflora (Absheron), and *Q.castaneifolia* (Absheron) (with 0.364). *Q. petraea* subsp. iberica (Ismayilli) and *Q. ilex* (Absheron) were considered the most distant genotypes in terms of genetic relationships.

Table 8 Jaccard similarity coefficient based on RAPD markers of oak taxa

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

7.4. Genetic kinship between genotypes. UPGMA dendrogram of 5 oak taxa was constructed using RAPD marker data based on the Jaccard similarity coefficient (Figure 2). Dendrogram results showed that the studied *Quercus* taxa could be completely divided into three main groups based on the values of the similarity coefficient between 0.333 and 0.818. In the study, the cophenetic correlation coefficient between the similarity matrix and the UPGMA dendrogram was r=0.861. A coefficient greater than 0.80 indicates that the dendrogram is perfect. Based on a cluster analysis of biomorphological and genetic data of oak genotypes, *Q. macranthera* subsp. *macranthera*, *Q. robur*

subsp. *pedunculiflora* (K. Koch) Menitsky, *Q. petraea* subsp. *iberica* taxa were found to be genetically close to each other.

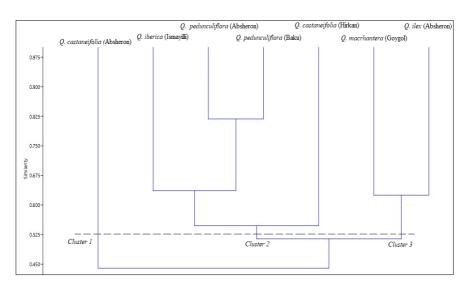


Figure 2. UPGMA dendrogram of the RAPD analysis of *Quercus* taxa based on the Jaccard coefficient

Genetic relationship between oak taxa was studied by Principal Coordinate Analysis (PCoA). The results obtained using RAPD markers were subjected to Principal Coordinate Analysis based on the Jaccard similarity coefficient. The 2D plot of PcoA obtained based on the results of RAPD analysis consists of 3 main groups (Figure 3.). PCoA identified the first two most informative coordinates, 0.328 and 0.222, representing 73.48% of the total genetic variation. PCoA results confirmed the results of the UPGMA Cluster analysis. Based on the results of the Principal Coordinate Analysis, it can be concluded that the RAPD analysis revealed significant polymorphisms in some oak taxa in Azerbaijan²⁴.

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²⁴ Aliyeva, G. Molecular diversity and phylogeny analysis of Azerbaijan oaks (*Quercus* spp.) revealed by RAPD markers / G. Aliyeva, J.Ojaghi, S.Rustamova // Caspian Journal of Environmental Sciences -2021. Vol. 19. No. 3. – pp. 457-468.

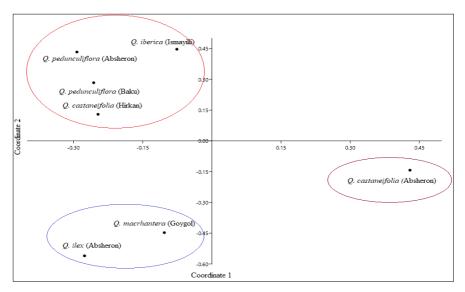


Figure 3. Principal coordinate analysis (PCoA) showing Jaccard genetic similarity between oak taxa under the study

High polymorphism in molecular genetic research in oak taxa is an indication of high genetic diversity. This suggests that local oak taxa have a high potential for the emergence of new taxa, which is of particular importance for the preservation of these taxa in the flora. High values of EMR and MI used for the evaluation of genetic changes and the determination of the phylogenetic relationship between differrent Quercus taxa with a small set of primers at the level of sufficient polymorphism, phylogenetic analysis of oak populations show that the RAPD markers used are useful in molecular identification of taxa. The data from this study can be of practical use for mapping the oak genome and classical selection. This study will be useful in future research as a preliminary study to determine the level of intra-taxa and inter-taxa genetic diversity and to select hybrids among taxa. We hope that the results of the study can make a significant contribution to setting effective management strategies for the protection of the valuable gene fund of *Quercus* in the Caucasus.

CONCLUSIONS

- 1. Based on the analysis of variance (ANOVA) of morphological polymorphism caused by various environmental factors in different oak taxa from various regions of Azerbaijan, the level of intra-population polymorphism was higher than that of inter-population polymorphism. Traits with the highest variation are leaf width/length ratio (CV=110.03%), shape factor (CV=53.12%) and leaf area (CV=34.01%).
- 2. As a result of the Principal Component Analysis (PCoA) of morphological traits, 3 indicator elements (PC1, PC2, and PC3) explained 86.97% of the whole variations between genotypes. Traits such as leaf length and leaf width/length ratio were found to be the determinants for the taxa *Q.robur* subsp. *pedunculiflora*, *Q.petraea* subsp. *iberica* və *Q.macranthera* subsp. *macranthera*, leaf area, leaf width, and shape factor for *Q. castaneifolia*, and the leaf width/length ratio-for the taxa *Q.ilex*.
- 3. In oak taxa, the physiological parameters (Leaf Mass per Area, Water Content, Relative Water Content, Juiciness) of the leaf positively correlated with the average annual precipitation and altitude, but negatively correlated with the average annual temperature. Changes in these indicators positively correlated with changes in leaf sizes especially changes in leaf area, which is the main adaptability indicator in plants.
- 4. Based on the statistical analysis of the morphometric results, the status of three oak taxa in the local flora was specified: eastern oak *Quercus macranthera* subsp. *macranthera* (K.Koch) Menitsky, Georgian oak *Quercus petraea* subsp. *iberica* (Stev.), pedunculate oak *Quercus robur* subsp. *pedunculiflora* (K.Koch.) Menitsky.
- 5. Eight of the applied RAPD primers could be used as specific markers in the identification of local oak taxa (22 specific bands), while with the other two primers (OPB-04 and OPD-08) no specific bands were identified for any taxon. The most specific band was synthesized using OPD-11 primer. The highest number of polymorphic bands was observed with the OPB-02 primer, and the lowest number of polymorphic bands was observed with the OPB-05 primer.

- 6. The average value of genetic polymorphism in the studied oak taxa was equal to 79.38%. The variation of the Jaccard similarity coefficient between 0.333 and 0.818 indicates that the genotypes are significantly different from each other and the potential for adaptability and new taxa formation is high in the studied taxa.
- 7. As a result of the cluster analysis, the genotypes were grouped into seven clusters according to the biomorphological characteristics of the oak genotypes, and into three clusters according to the genetic data. Both analyses allowed us to conclude that *Q.robur* subsp. *pedunculiflora* and *Q. petraea* subsp. *iberica* taxa are phylogenetically closer to each other.

RECOMMENDATIONS

- 1. It is proposed to plant new oak forests, restore oak forests and biodiversity in Karabakh, and collect acorns suitable for planting in autumn months for the greening of parks and alleys in cities. If necessary, such seedlings can be easily transferred to the appropriate areas.
- 2. The widespread use of evergreen Holm oak in the landscaping of parks and alleys and the establishment of new forests by transferring the taxa to the natural flora would make a great contribution to our ecology and application of the "truffle business" in these forests would develop our economy.
- 3. To create a gene bank of endemic oak taxa for the Caucasus existing in the flora of Azerbaijan.

List of published scientific works on the topic of the dissertation:

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