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EVALUATION OF MORPHOLOGICAL TRAITS AND GENOTYPES BY MULTIVARIATE STATISTICAL METHODS IN SOME OAK SPECIES

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ОЦЕНКА МОРФОЛОГИЧЕСКИХ ПРИЗНАКОВ И ГЕНОТИПОВ МНОГОМЕРНЫМИ СТАТИСТИЧЕСКИМИ МЕТОДАМИ У НЕКОТОРЫХ ВИДОВ ДУБА

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Abstract. In this study, evaluated some morphological traits and genotypes by multivariate statistical methods in some oak species (*Q. castaneifolia* C. A. Mey, *Q. pedunculiflora* C. Koch., *Q. iberica* Stev., *Q. macranthera* Fisch. & C. A. Mey ex Hohen, *Q. ilex* L.). 910 leaves were sampled from 91 trees, 8 population across Azerbaijan, and 6 morphological traits were assessed. The indicator traits were analyzed using multidimensional statistical analysis for each species. As a result of the component analysis, the three-pointer element (PRIN1, PRIN2, PRIN3) explained 86.97% of the variance among genotypes. These results provide identification of valuable species and patterns in the future selection and application of other genetic programs on the improvement of oaks in Caucuses.

Аннотация. Проведена оценка некоторых морфологических признаков и генотипов с использованием многомерных статистических методов у некоторых видов дуба (*Q. castaneifolia* C. A. Mey, *Q. pedunculiflora* C. Koch., *Q. iberica* Stev., *Q. macranthera* Fisch. & C. A. Mey ex Hohen, *Q. ilex* L.). Было отобрано 910 листьев с 91 деревьев, обследовано 8 популяций из Азербайджана и оценены 6 морфологических признаков. Индикаторные признаки были проанализированы с помощью многомерного статистического анализа для каждого вида. В результате компонентного анализа три элемента-указателя (PRIN1, PRIN2, PRIN3) охватили 86,97% дисперсии между генотипами. Эти результаты позволяют идентифицировать ценные виды и образцы для будущего отбора и применения других генетических программ по улучшению дуба на Кавказе.

Keywords: Quercus, leaf morphology, population, variability, ANOVA, PCA.

Ключевые слова: Quercus, морфология листьев, популяция, изменчивость, ANOVA, PCA.

Introduction

The genus *Quercus* L. (Fagaceae) is a diversified group of temperate trees with about 500 species distributed worldwide [1]. *Quercus* is one of the most important woody genera of the Northern hemisphere and considered as one of the main forest tree species in Azerbaijan [2]. The oak has a special symbolic, ecological and economical value in Azerbaijan.

Forest trees, like oaks, rely on high levels of genetic variation to adapt to varying environmental conditions. Thus, genetic variation and its distribution are important for the long-term survival and adaptability of oak populations [3–4]. Plant taxonomists believe that the leaves of some oak species under environmental change and habitat factors such as elevation change or altitudinal gradients show different morphological forms; therefore, several dichotomous keys based on morphological characteristics have been developed to describe species and sections within Quercus [1, 5].

Differences in phenotypic and physiological responses are associated with the geographical locations of populations at local or regional scales. Leaves are organs that are exposed to different environmental factors, and it is reasonable to expect that their morphology and structure represent the responses of the plants to local conditions, such as water availability or light intensity, as well as intra- and interspecific interactions [6–8].

The study of leaf morphology from the aspect of genetic differentiation provides useful information on population and intrapopulation variability and can be the basis for the determination of species and lower categories as well as intraspecific or interspecific hybrids. The similarity between individuals of the same or different populations or between distant and separate populations can point to their historical connections and common descent. Morphological determination is a good basis for further studies of this kind, and it is often combined with chemotaxonomic, cytological and molecular analyses [4, 9–10].

Using the geometric morphometric approach, shape variability is studied as a geometric property of leaves without any effect of size, and thus, morphometrics provides a powerful tool for exploring shape differences among taxa and for investigating intraspecific variability due to genotypic differences and phenotypic plasticity. In fact, new morphometrics are useful for quickly generating and managing large amounts of phenotypic data representing many aspects of the phenotype [11–12]. The protocol for studying leaf morphology in oaks [13] revealed significant differences within and between individuals, populations and species in particular, when a mean leaf shape for each tree was analyzed, the differences between populations and between species were highly significant. In fact, the use of PCA represents a useful procedure for extracting new, uncorrelated variables for describing the variation in discrete shape traits. The shape variation can be visualized and described along the scores of each PC, and its heritability can be tested by univariate statistical analyses (i.e. ANOVA), while multi-variate statistical analyses such as MANOVA / CVA detect cumulative effects of the shape traits in species differentiation [14].

The previous researches on leaf morphology of Azerbaijan oaks were generally conducted by traditional methods [15–19]. In this research, for the first time, the macromorphological properties of oak leaves were measured using modern methods and equipment, and the results were analyzed by statistical analysis. It is a part of a larger study on the ecological, morphological, and molecular characterization of these five species in Azerbaijan. The main goals of the study are:

1) To collect comparative morphological data of some species of the *Quercus* genus in the country.

2) Assessment of characters and genotypes using multivariate statistical methods.

3) Identification of valuable species and patterns in the future selection and application of

other genetic programs.

4) Identify descriptor traits for each studying species.

Materials and methods

Study populations. Study species were *Q. castaneifolia* C. A. Mey., *Q. pedunculiflora* C. Koch., *Q. iberica* Stev., *Q. macranthera* Fisch. & C. A. Mey. ex Hohen**.** Plants were collected in diverse forest types between −28 and 2200 m in elevation in Azerbaijan. The geography and ecology of these areas are given in Table 1. 91 tree specimens (*Q. castaneifolia* C. A. Mey., *Q. pedunculiflora* C. Koch., *Q. iberica* Stev., *Q. macranthera* Fisch. & C. A. Mey. ex Hohen, *Q. ilex* L.) were chosen [6] from 8 inhabitants of *Quercus* trees around Azerbaijan (Table 1) in 2017. Chestnut-leaved oak (*Q. castaneifolia*) leaf samples were collected from Hirkan National Park (HNP) — Astara, Lankaran plain (LP) and Mardakan arboretum (MA) (56–63). Georgian oak (*Q. iberica Stev*.) (11–20) leaf samples were collected from Ismailli. The study areas of pedunculate oak (*Q. pedunculiflora*) were Baku (Botanical garden) (87–91), Absheron (Mardakan arboretum) and Ganja**.** Caucasian oak (*Q. macranthera*) leaf samples belong to Goygol National Park. And finally, holm oak (*Q. ilex l.*) leaf samples were gathered from Baku (Botanical garden and Officers' Park and Absheron (Mardakan arboretum). The same sampling design and methods were applied for each population. 10 mature trees of small area (0.5–1.0 ha) of homogeneous open oak forest were selected. 8–10 m tall trees were chosen and four outermost branches (light subsample) and four innermost branches (shade subsample) of each tree crowns were randomly selected. To avoid seasonal and positional variations, samples were collected from different branches at approximately the same height and location, where leaf growth had stopped. Branches were collected from the four cardinal compass directions. The leaves' ages were practically the same, although there is a small variation in budburst among trees and within trees. In experimental design, only branch position considered [20–21]. The most important factor within-plant variation is inner vs outer position of branch regardless of compass direction or height.

Table 1.

GEOGRAPHIC LOCATION AND CLIMATE CONDITIONS OF THE SAMPLED OAK POPULATIONS [1, 22]

Locality	*Geographic coordinates*	*Altitude, m*	*Pa, mm*	*T, °C*
Baku	40°23′N 49°51′E	−28	990–1200	14.2
Absheron	40°33′N 49°30′E	8	180–300	14–15
Ismailli	40°35′N 47°45′E	500–800	500–1000	14.0–14.5
Gabala	41°25′N 47°23′E	900	800–850	10–12
Ganja	40°40′N 46°21′E	400–450	200–300	13.1
Goygol	40°37′N 46°34′E	1000–2200	500–900	13.5
Lankaran plain	39°24′N 48°58′E	−28–200	1280	14.1
Hirkan National Park	38°47′N 48°69′E	534	1200–1750	11–13

Pa — annual precipitation (millimeters); T — mean annual temperature, °C).

Morphometric analysis. The morphological study of the oak leaf included 10 leaf samples per tree, on 91 trees in 7 populations, which makes a total of 910 leaves (10 trees per population) [6, 13, 23]. Generally, 6 morphometric parameters were analyzed. The morphological characters utilized in this study:

LA (cm^2) — leaf area.
LL (cm) — leaf length.
LW (cm) — leaf width.
LP (cm) — leaf perimeter.
R — Ratio (R=LL/LW).
F — Leaf shape Factor.

Morphological traits were measured by CI-202 LESER AREA METER (USA) on ten leaves stripped of the petiole for each subsample. For each character, mean values of each population were calculated.

Statistical analysis. Two statistical tests namely KMO (Kaiser–Meyer–Olkin) and Bartlett were used for correctly performance of PCA. The most important data on population and individual variability were described by results of descriptive statistics. Species was treated, as a fixed variable; trees were considered as a random factor nested within species because trees were representative of each population. Statistical significance of different sources of variation, with the population as a fixed and the trees as a random factor, was determined by using the analysis of variance (ANOVA, SPSS 16, PAST and MSTATC). This analysis used only the characteristics that showed statistical significance as determined by the results of ANOVA.

Results and discussion

It is becoming increasingly clear that not only trait means, and genetic structure can vary within a species, but also phenotypic plasticity in those traits. Moreover, the mean value and the plasticity of a trait may interact [24]. From the perspective of assessing the contribution of plasticity to persistence and distributional shifts under climate change, it is the adaptive component that is of interest, i.e., plasticity that allows a genotype to maintain high fitness across environmental gradients [22, 25]. There is ample evidence, though, that populations within a species experiencing different environmental conditions often differ in phenotypic characters and genetic structure [26].

In this study, the relative importance of 6 morphological traits of leaves for each genotype were analyzed. It was demonstrated that environmental factors are associated with morphological variation in different oak species that occurs in different types of forests. It has been documented that the leaf morphological variability of species along elevational gradients is related to environmental factors [19].

The principal component method was used to investigate the importance of various traits in genotypes. Two statistical tests — KMO and Bartlett tests are used for the correct performance of principal component analysis statistically (Table 2). According to the results of these two tests, the KMO test value (0.58) and the statistical significance of the Bartlett test indicate that the "principal component" analysis was correctly implemented. As a result of the component analysis, three pointer elements explained 86.97% of the variance among genotypes (Table 3.). In the studied oak populations, each element effectively explained the interpopulation variations up to three-pointer elements (Figure). However, this variation began to decline sharply after three pointer elements. As a result, all analyses were performed on the basis of three selected pointer elements

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(PRIN1, PRIN2, and PRIN3). Table 3 shows the values of the pointer elements obtained based on the morphological characters. Table 4 reflects the values of indicator elements based on genotypes. It is possible to select effective genotypes based on one or more traits through the values of these elements. The results provided a clear description of the typical leaf shape of each species, and the differences between the species were evident when the mean contours were visualized and compared.

Table 2.

RESULTS OF KMO AND BARTLETT TESTS

Kaiser-Meyer-Olkin (Measure of sampling adequacy)		0.58
Bartlett's experiment	Xi square	265.27
The degree of exemption		15
Significance		0.000

Table 3.

RESULTS OF THE ANALYSIS OF COMPONENTS FOR EACH STUDIED TRAITS

Morphological characters	*PRIN1*	*PRIN2*	*PRIN3*
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

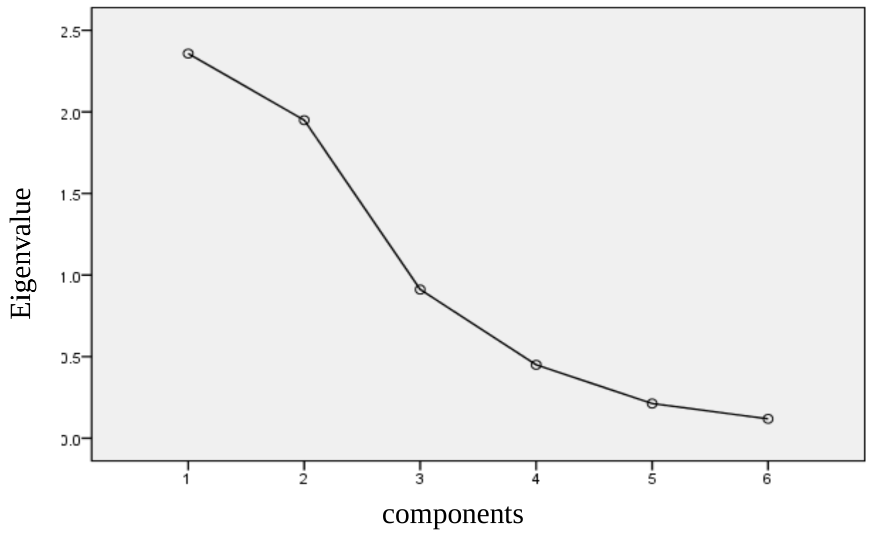

Figure. Scree plot based on analysis of components.

PRIN1 is significant because it explains 39.29% of the total variations (Table 3). R and LL were evaluated at maximum value in the current PRIN. Selection of valuable genotypes on the basis of the first indicator elements (it is clear that genotypes 11, 12, 20, 61, 67, 68, 72, 75, 78, 79, 80, 81, 82, 84, 85, 86 and 87 are highly valued for PC1 (Table 4.) will bring about the development of traits such as R (LL/LW) and LL in these genotypes. The second indicator element (PRIN2) was explained 32.49% of the total variation (Table 4). Significant traits in this PRIN were LA, LW, and F. The most valuable genotypes for the second indicator element (PRIN2) were genotypes 12, 42, 44, 45, 47, 49, 61, 70, and 73. The third indicator element (PRIN3) contains 15.9% of the total variation. LL, LW, and P traits were the most important traits in these PRIN. The most valuable are genotypes 39 and 52 in the current PRIN. This creates ample opportunities for the use of current materials as appropriate parental forms in future breeding and other genetic programs.

We found that leaf length and ratio are the most discriminating leaf descriptor between *Q. macranthera, Q. iberica* and *Q. pedunculiflora*. Leaf area, leaf width and factor are significative morphological traits for *Q. castaneifolia*. Leaves of holm oak are smaller than other studied species. Ratio (leaf length / leaf width) may be descriptor for holm oaks (*Q. ilex*).

Multivariate analysis of variance provides an important tool for visualizing the morphological traits that characterize this species complex and play a notable role in the identification and systematics of this plant species. These results provide identification of valuable species and patterns in the future selection and application of other genetic programs on the improvement of oaks in Caucuses.

Table 4.

VALUES OF INDICATOR ELEMENTS IN ACCORDANCE WITH STUDIED GENOTYPES

Genotypes	*PC 1*	*PC 2*	*PC 3*	*Genotypes*	*PC 1*	*PC 2*	*PC 3*
1	0.01	−1.59	−0.17	49	−1.73	2.65	−0.64
2	0.02	−1.52	0.10	50	−1.11	0.64	−0.28
3	0.03	−0.46	−0.10	51	−2.46	0.21	−0.60
4	−0.28	−1.49	0.01	52	−4.45	1.17	5.32
5	−0.06	−1.52	0.07	53	−1.17	0.44	−0.36
6	1.26	−0.85	0.39	54	−0.98	1.36	−0.37
7	0.52	−1.17	0.17	55	−1.46	1.62	−0.45
8	0.15	−1.60	0.16	56	0.31	0.62	−0.17
9	0.52	−1.09	0.04	57	−0.74	−0.18	−0.39
10	−0.39	−1.72	−0.06	58	−0.68	−0.49	−0.45
11	1.40	−0.33	0.60	59	0.73	1.13	−0.07
12	2.11	1.83	0.46	60	−0.59	−0.61	−0.21
13	1.40	0.46	0.37	61	1.47	1.65	0.31
14	−0.67	0.25	−0.21	62	0.01	0.74	−0.18
15	0.96	0.27	0.17	63	−0.81	−0.01	−0.37
16	0.15	−0.41	−0.10	64	−0.41	−0.51	−0.31
17	0.04	−0.40	0.15	65	−0.16	−0.26	−0.54
18	0.27	0.32	−0.02	66	0.33	−0.22	−0.46
19	0.34	−1.14	0.20	67	2.44	1.38	0.48
20	1.52	0.50	0.30	68	1.85	0.10	0.39
21	−1.28	−2.59	−0.14	69	0.57	−0.11	−0.03
22	−1.18	−1.32	−0.58	70	−0.73	2.18	−0.58
23	−1.05	−1.86	−0.28	71	0.81	0.471	−0.25
24	0.25	−3.25	0.18	72	2.44	1.38	0.48
25	0.48	−1.72	−0.15	73	1.10	2.37	−0.26
26	0.31	−2.06	−0.14	74	−0.23	0.66	−0.66
27	0.10	−2.44	−0.03	75	1.93	1.34	0.10

Genotypes	PC 1	PC 2	PC 3	Genotypes	PC 1	PC 2	PC 3
28	0.36	−2.024	0.04	76	−1.23	1.32	−1.16
29	0.17	−2.05	−0.04	77	1.08	0.24	0.30
30	0.41	−1.48	−0.07	78	1.63	0.84	0.39
31	0.35	−1.22	−0.12	79	1.71	0.74	0.34
32	0.15	−2.02	−0.09	80	2.38	0.48	0.61
33	−0.23	−2.70	−0.09	81	1.62	1.08	0.27
34	0.58	−1.50	0.09	82	2.81	1.48	0.56
35	−0.31	−2.33	−0.15	83	0.26	−0.92	0.04
36	−1.85	−0.59	−0.19	84	1.64	−0.37	0.38
37	−2.32	1.27	−0.74	85	1.99	−0.04	0.36
38	−2.32	−0.06	−0.64	86	3.32	2.96	0.62
39	−4.02	0.72	5.88	87	2.04	2.67	−0.28
40	−2.00	0.02	−0.71	88	1.36	0.18	−0.06
41	−3.42	0.93	−1.46	89	0.67	0.11	−0.26
42	−4.12	1.97	−1.46	90	0.85	0.66	−0.16
43	−2.97	0.59	−1.28	91	0.11	−0.62	−0.31
44	−1.87	2.50	−1.10				
45	−2.39	2.48	−1.02				
46	0.33	−0.11	0.33				
47	−0.29	2.07	−0.03				
48	0.33	−0.11	0.33				

1–10 *Q. ilex* (Absheron), 11–20 *Q. iberica* (Ismayilli), 21–25 *Q. ilex* (Baku 1), 26–35 *Q. ilex* (Baku 2), 36–45 *Q. castaneifolia* (Hirkan), 46–55 *Q. castaneifolia* (Lankaran), 56–63 *Q. castaneifolia* (Absheron), 64–66 *Q. pedunculiflora* (Absheron), 67–76 *Q. pedunculiflora* (Ganja), 77–86 *Q. macranthera* (Goygol), 87–91 *Q. pedunculiflora* (Baku).

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