

## Morphological characteristics of seed of the endangered species of *Diospyros lotus* (Ebenaceae) in Azerbaijan

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**Abstract:** Plants are one of our main food sources, and also include bioactive compounds for maintaining human health. *Diospyros lotus* L. is an economically important species with edible fruits and valuable timber. The leaves, fruits and seeds of the plant are used in folk medicine. The aim of this research was to study morphological characteristics of seed and seed weight of *D. lotus*, collected from natural ranges of the species in the northwest and southeast of Azerbaijan in 2016, 2018 and 2020. We have selected five morphological characteristics of seed: length, width, area, minimum circumscribed, and perimeter. The results showed that most of the seeds examined were medium sized with the following length, width and area ratios: 9.26-11.65 x 5.05-6.44 x 42.00-63.81 mm. Fruit size in the population varied from 2.2 cm to 0.97 cm. Moreover, *D. lotus* is a nationally endangered species within its natural habitat, and is included in the Red Book of Azerbaijan. Given the current decline in wild populations of this species across its natural range, both regionally and globally, implementation of appropriate studies is important. Morphological variability and differences between seeds can directly influence their ability to germinate, which is very important for the conservation of the species. This study could also support the analysis of taxonomic relationships to increase understanding of the phylogeny and status of *D. lotus*.

**Key Words:** *dateplum*, *fruit weight*, *Lankaran*, *Qakh*, *seed shape*, *wild species*

### INTRODUCTION

Seed is a main genetic source and dispersal method between current and future generations and populations of plants. Morphology studies are often required in various research fields, such as agronomy, genetics, ecology and taxonomy [Iwata, Ukai, 2002; Segarra, Mateu, 2001]. Seed shape can vary with species, seed maturity and due to environmental conditions, chemicals or disease [Morozowska, 2011].

*Diospyros lotus* L. (English: Dateplum, Caucasian persimmon; Azerbaijan: *Adi xurma*) is a deciduous tree 15 or rarely 20 m tall, from the family Ebenaceae. Wild populations grow in the northwest, northeast and southeast of Azerbaijan. It has been cultivated for edible fruits mainly in the lowlands, foothills and lower mountain zone in the southeast, northwest and northeast of the country [Flora of Azerbaijan, 1957, VII:62-66; Mehdiyeva et al., 2016]. It occurs in mixed forests of the lower and middle mountain zones up to 1100 m above sea level.

It is reported that the flora of Azerbaijan includes 467 species of trees and shrubs [Flora of Azerbaijan, 1950-1961]. According to recent studies, about 189 existing species need protection [Qurbanov et al., 2015]. In Azerbaijan, *D. lotus* is an endangered species listed in the Red Book of Azerbaijan, with the category VU D2 (IUCN) [Red Book of Azerbaijan, 2013]. It is also included in the IUCN database with LC category for its global natural distribution, as the number of individuals of this species is already declining globally within its natural range. Dateplum is propagated in the wild mainly by seeds. Research on the seeds of this species is important for its conservation.

*D. lotus* has several useful properties e.g. ornamental, nutritional, melliferous and medicinal, which can be widely used in landscaping and afforestation [Flora of Azerbaijan, 1957; Ibadullayeva, 2013; Mehdiyeva, 2011; Ali-zade et al., 2019]. Its seeds comprise 2.6% oil and 9.3% protein by content [Barclay, 1974]. Leaves are used for the treatment of stroke and apoplexy syndrome in traditional Chinese Medicine and as a hypotensive drug in Japan [Fan, He, 2006]. The seeds are used for

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Received 30.10.2020; Received in revised form 30.11.2020; Accepted 02.12.2020

their sedative property in China [Abdur et al., 2015]. In addition, it has been used as herb tea for the treatment of hypertension in patients in Korea [Sun, 2011]. In Azerbaijan, especially in the Talish region, pulp of the fruits is applied to furuncles and wounds; fresh and processed fruits are used to treat anemia, and diseases of the liver. Fruits are used in food in fresh, dried and processed form. Honey from *D. lotus* is yellow, thick and sweet [Mehdiyeva et al., 2016]. Moreover, the bark leaves and fruits are used to obtain orange and beige dyes [Qasimov, Muradov, 2017].

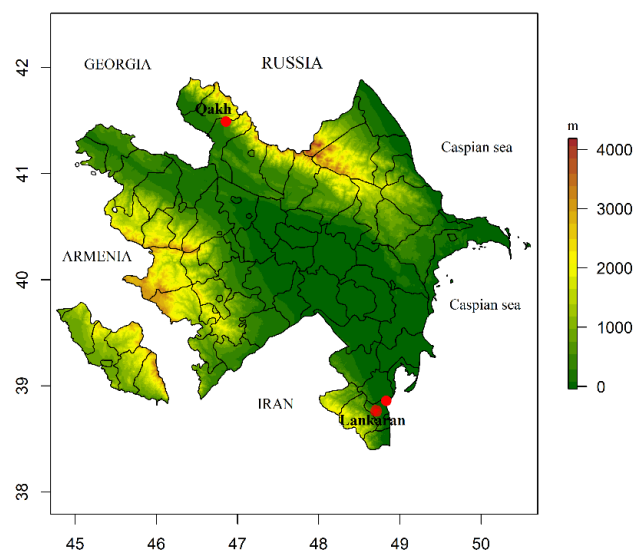
A number of studies have been conducted to determine the morphological characteristics of seeds of *D. lotus* L. Many research studies have focused on morphological features and color characteristics of the species using seed images [Li, 2017], size, shape and color of mature fruits [Utsunomiya et al., 1998], morphological analysis, quantitative and qualitative characteristics of buds, leaves, flowers, fruits and seeds [Grygorieva et al., 2009]. In Azerbaijan, studies have mainly focused on useful features of this species: as a dye [Qasimov, Muradov, 2017], food [Ibrahimova, 2019] and medicine [Mehdiyeva, 2011; Mehdiyeva et al., 2016].

The present study aims to describe the morphological characters and weight of seed samples of *D. lotus*, collected from its wild populations. The following aspects were explored: variation between seed samples, analysis of the similarities and differences in seed morphological features, determination of the relationship among seed collections. The article also provides information about the fruit weight and shape of *D. lotus*.

## MATERIAL AND METHODS

**Study area.** Azerbaijan is located in the east of the South Caucasus, at the crossroads of Eastern Europe and Southwestern Asia (Fig. 1). The total area of the country is 86.600 km<sup>2</sup>. The elevation changes over a relatively short distance from lowlands at 27 m below sea level to highlands up to 4466 m a.s.l. [Museyibov, 1998]. Qakh district, with an area of 1494 km<sup>2</sup> is situated in the northwestern part of the country and the southern part of the Greater Caucasus. According to the botanical-geographical region of Azerbaijan, the northern and northeastern parts of the Qakh district belong to the western Greater Caucasus, the central part to the Alazan-Ayrichay valley, and the southern part to Bozgir plateau botanical-geographical region [Flora of Azerbaijan, 1957]. Forest covered areas are

mainly located in the northern and north-eastern part of the district; approximately 50% of the central part and small areas of the southern part are covered with forests. The climate of the region is dry subtropical in the southern part, moderately hot in the central part, semi-humid subtropical, cold in the highlands. Annual precipitation is 300 mm in the south and 1600 mm in the north [Mammadov et al., 2010; Museyibov, 1998].



**Figure 1.** Location map of Azerbaijan; red circles indicate the wild populations of *Diospyros lotus*, where seeds were collected (Qakh and Lankaran districts).

Lankaran district is located in the south-east part of the republic. It is bounded on the north by the Kur-Araz lowland, and in the southeast of the Caspian Sea (Fig. 1). Lankaran has a humid subtropical climate and a hot-summer Mediterranean climate. It has cool, wet winters and very warm, partially dry and highly humid summers. Lowland vegetation is predominantly meadows, while the mountains and foothills are forested. About 14% of the total area of the district is covered with forest. Maximum annual precipitation lies between 1600-1800 mm, which is the highest precipitation rate in Azerbaijan [Mammadov et al., 2010; Museyibov, 1998].

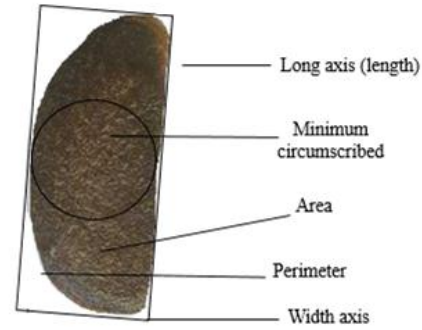
**Study species.** In Azerbaijan *D. lotus* grows naturally in the Eastern Greater Caucasus, Western Greater Caucasus, Samur-Devechi lowlands, Lankaran plain and mountain parts, Bozgir plateau, and Alazan-Ayrichay valley botanical-geographical regions [Askarov, 2011; Flora of Azerbaijan, 1957]. It has also been cultivated for its edible fruits. Fruit is globose, has a 10-20 mm diameter, 1-10 seeded fleshy berry, with a yellow or bluish-black colour at maturity. *D. lotus* flowers in May

and June, and fruits between October and November. Dateplum forests mainly grow on humid slopes, with rich humus and well-moistened soil. Reproduction is by seeds and spears [Flora of Azerbaijan, 1957; Mehdiyeva et al., 2016].

Seeds were collected from the wild populations of the species in Burjali village of Lankaran district (36 m a.s.l.) on 14.10.2016, close to Lankaran district on 19.09.2020, Lekit village (947 m a.s.l.; 894 m a.s.l.) of Qakh district on 19.10.2016 and 27.11.2020. The seed collections are stored in the Seed Bank of the Institute of Botany, ANAS in Azerbaijan and at the Millennium Seed Bank, Royal Botanic Gardens, Kew in the UK. The herbarium vouchers are deposited in the Herbarium of the Institute of Botany (BAK) and Herbarium of the Royal Botanic Gardens, Kew.

**Morphological measurements.** Morphological seed characters of *D. lotus* were determined using 100 mature seeds from each population. The seed samples were scanned using an Epson Perfection scanner V700 and each sample was measured with ImageJ 1.x software [Schneider et al., 2012; Medina et al., 2010; Tanabata et al., 2012; Williams, 2013]. The morphological measures were extracted from the images of individual seeds. The values were obtained directly with ImageJ. We used five dimensions for the description of morphological characters as shown in table 1 and figure 2 [Li, 2017]. The weight of seeds and fruits was determined by Adam PW254 analytical balance.

**Statistical analysis.** To analyze seed samples, we divided total samples from all populations into three seed sizes categories: small, medium, and large. For interval estimation, we calculated the range of each morphological seed characters, which is the difference between the largest and smallest values in the data set. Then obtained value was divided equally into three classes and upper and lower boundaries of the interval were determined. A correlation analysis was performed to study the relationship between the seed length, width,



**Figure 2.** The morphological characters used in the analysis of seed samples of *Diospyros lotus*.

area, minimum circumscribed and perimeter. We also used regression analysis to determine the relationship between seed length and width, and seed length and area, using a parametric Pearson's coefficient ( $r$ ). Statistical analyses were performed using R version 4.0.2. [R Core Team, 2019].

**Ecological associations.** The predominant species in the ecological association of each population was identified by using the route and sampling methods [Field geobotany, 1960]. The accepted scientific plant names were determined based on the Flora of Azerbaijan, then verified and updated according to other sources [WFO, 2020].

## RESULTS AND DISCUSSION

**Seed morphological characters.** For a better analysis of the seed collections of *D. lotus*, we divided the samples into three seed sizes categories: small, medium, and large (Tab. 2). Most of the seeds examined were medium size (Fig. 3). Thus, based on length measurements, 60.75% of the total seed samples are medium-sized, while this is also reflected in the results of width and area measurements, with 57.25% and 53.50% respectively. Large seed sizes were the least represented by the samples studied. Based on the results of length,

**Table 1.** A morphological characters descriptor used in the analysis of seed samples of *Diospyros lotus*.

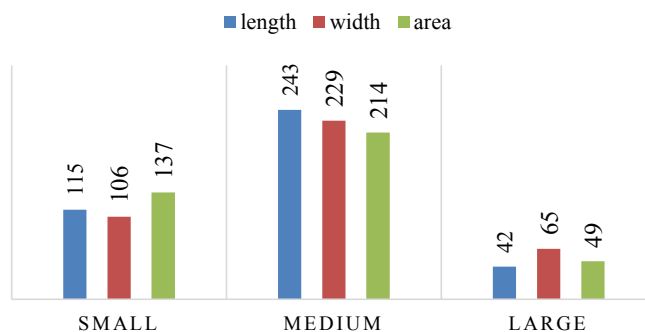
Morphological characters	Description of morphological characters
Long axis (length)	the length of the minimum bounding box the seed boundary, represented by the length of the smallest rectangle containing the seed
Width axis (width)	the width of the minimum bounding box of the seed, represented by the width of the smallest rectangle containing the seed
Area	the width of the minimum bounding box of the seed, represented by the width of the smallest rectangle containing the seed
Perimeter	the sum of the Euclidian distances of successive points on the polygon
Minimum circumscribed	the radius of the smallest surrounding circle of a seed

**Table 2.** Seed size categories used in the study.

Seed size	Morphological characters (mm)		
	length	width	area
Small	6.89-9.26	3.66-5.05	20.19-42.00
Medium	9.27-11.65	5.06-6.44	42.01-63.81
Large	11.66-14.04	6.45-7.84	63.82-85.62

width, and area measurements about 10.5%, 16.25% and 12.25% of the total number of seed samples are small-sized.

Overall, seed shape range represented from a minimum of 6.881 mm to a maximum of 14.034 mm in length, 3.664–7.835 mm in width, 20.196–30.637 mm in area, 8.236–21.619 mm of minimum circumscribed, and 19.952–32.392 mm in perimeter (Tab. 3). Variation in seed size among samples from Qakh district (2020) is relatively high on length, width and area, with CVs of 10.33%, 11.29%, and 19.45% respectively. However,



**Figure 3.** Number of total collected seed samples by indicating seed size categories with the following length, width, and area ratios.

in 2018, seed samples collected in Qakh have a higher coefficient of variation on minimum circumscribed. In addition, seed samples differed from each other in shape (Fig. 4).

**Table 3.** Ranges, mean values, standard deviation, and coefficients of variation of seed morphological features of *Diospyros lotus* in Azerbaijan.

Characters (mm)	Seeds (n)	Min.	Max.	Mean (±Standart error)	Standart deviation (SD)	Coefficient of variation (CV%)
Lankaran, 2016						
Length	100	8.444	12.315	10.90901 (0.07463678)	0.7463678	6.841755
Width	100	4.632	7.468	6.21948 (0.05930365)	0.5930365	9.535146
Area	100	30.637	76.59	56.74089 (0.8788682)	8.788682	15.48915
Minimum circumscribed	100	15.897	40.865	27.07221 (0.4874854)	4.874854	18.00686
Perimeter	100	24.322	36.855	31.92978 (0.258917)	2.58917	8.10895
Length/width ratio	100	1.484168	2.183619	1.76316 (0.01375167)	0.1375167	7.799444
Qakh, 2018						
Length	100	7.775	11.087	9.50443 (0.07026128)	0.7026128	7.392477
Width	100	3.894	6.733	5.36606 (0.05995631)	0.5995631	11.17325
Area	100	23.377	65.037	42.9433 (0.7575821)	7.575821	17.64145
Minimum circumscribed	100	8.251	28.012	19.22016 (0.4049934)	4.049934	21.07128
Perimeter	100	21.97	39.561	27.81951 (0.294785)	2.94785	10.59634
Length/width ratio	100	1.392436	2.230992	1.785214 (0.01657198)	0.1657198	9.282911
Lankaran, 2020						
Length	100	6.881	10.915	9.0383 (0.07745409)	0.7745409	8.569542
Width	100	3.664	5.966	4.80446 (0.04644178)	0.4644178	9.666388
Area	100	20.196	49.02	34.9843 (0.6199629)	6.199629	17.72118
Minimum circumscribed	100	8.236	21.619	14.79898 (0.2687345)	2.687345	18.15899
Perimeter	100	19.952	32.392	25.9074 (0.2266409)	2.266409	8.748115
Length/width ratio	100	1.536284	2.258105	1.888872 (0.01470178)	0.1470178	7.783368
Qakh, 2020						
Length	100	8.134	14.034	10.91643 (0.1128064)	1.128064	10.33363
Width	100	4.197	7.835	5.95476 (0.06725853)	0.6725853	11.29492
Area	100	30.004	85.617	56.92472 (1.107643)	11.07643	19.45803
Minimum circumscribed	100	13.344	40.684	27.08201 (0.5216383)	5.216383	19.26143
Perimeter	100	22.181	37.532	30.69675 (0.3155632)	3.155632	10.28002
Length/width ratio	100	1.533353	2.485041	1.84246 (0.01683145)	0.1683145	9.135311



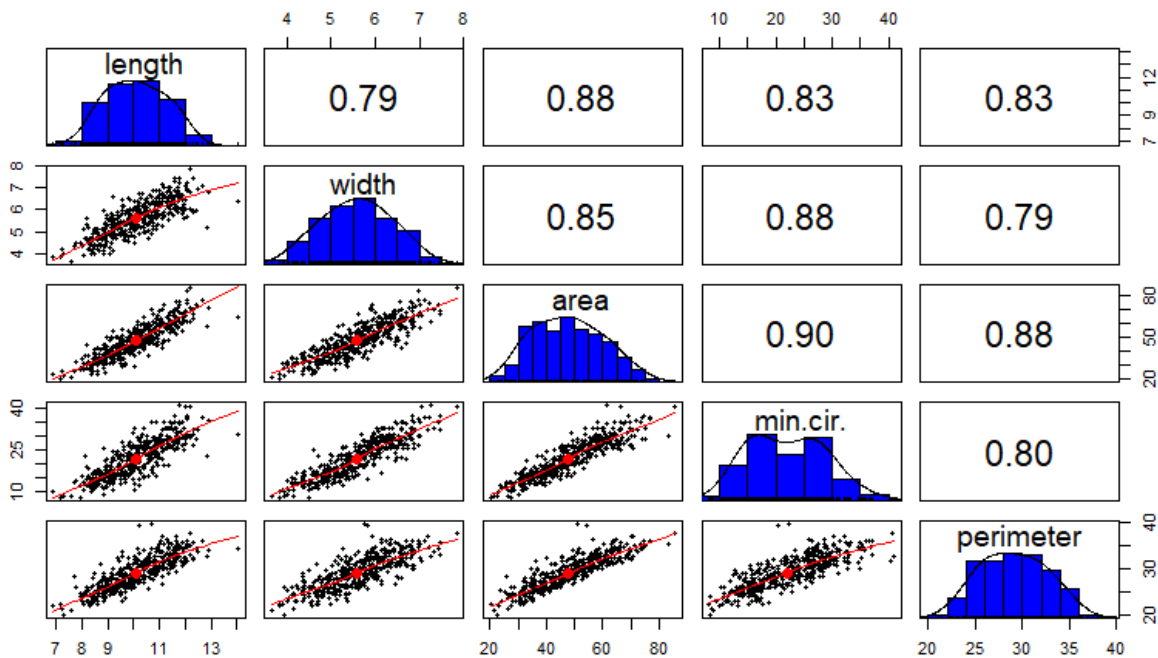
**Figure 4.** Variability of seed shape in the population of *Diospyros lotus* in Azerbaijan.

The seeds collected from Lankaran district in 2016 differ significantly from other seeds in size. Seed samples were analyzed for size and it was found that 3% of them were in the range of 8.44-9.21 mm in length, 6% in the range of 9.21-10.00 mm, and 91% in the range of 10.00-12.31 mm. Most of the seeds collected in Lankaran this year belong to the long category within the collection. Seed samples were also analyzed for their width and 4% of them were in the range of 4.63-5.19 mm, 19% in the range of 5.19-5.76 mm, 66% in the range of 5.76-6.9 mm, and 10% in the range of 6.9-7.46%. As a result, it was found that Lankaran seeds (2016) had a larger number of long specimens within the collection, but this did not significantly increase the width of 10% of the sample specimens.

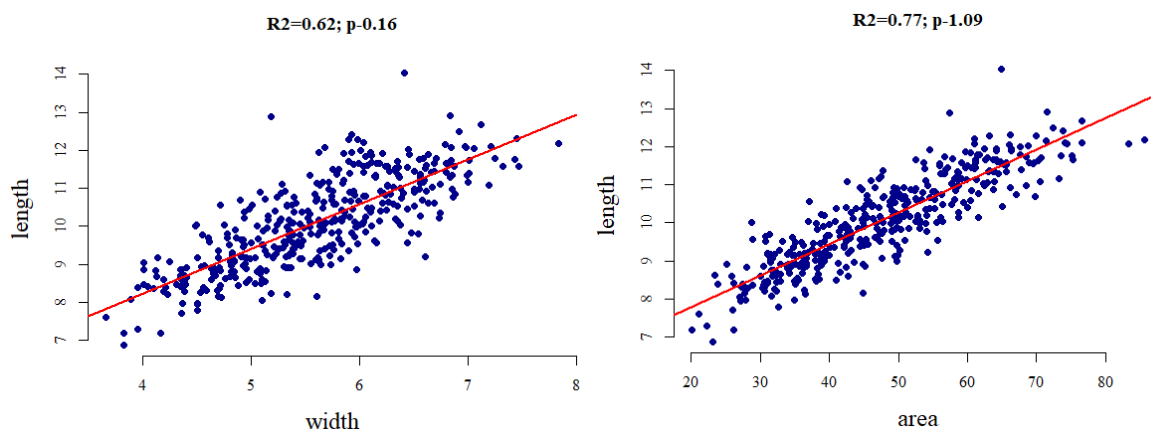
Generally, seed length, width, area, minimum circumscribed and perimeter were positively correlated

with each other (Fig. 5). Seed area was significantly positive correlated with seed minimum circumscribed ( $R=0.90$ ). Length and width, the two main features of seed size, were also positively correlated with each other ( $R=0.79$ ). Thus, as the length of our seed samples increases, seed width also increases in size. As a result, seed area, minimum circumscribed and perimeter also grow. This can be explained by the fact that seed area, minimum circumscribed and perimeter directly depend on the size of its width. There is a high correlation coefficient of seed width with seed area ( $R=0.85$ ), seed minimum circumscribed ( $R=0.88$ ), and seed perimeter ( $R=0.79$ ).

The length and width of a seed are considered one of its main morphological features. To investigate this, we carried out regression analysis on these features (Fig. 6). The results showed that the length of the



**Figure 5.** Correlation between seed length, width, area, minimum circumscribed and perimeter, with indicating of bivariate scatter plots (below the diagonal), histograms (on the diagonal) and the Pearson correlation coefficient (R) (above the diagonal).



**Figure 6.** Regression analysis between seed length, seed width, and seed area.

total seed samples had a positive relationship with the width ( $R^2=0.62$ ,  $p=0.16$ ). Seed length and seed area also showed a positive relationship between each other ( $R^2=0.77$ ,  $p=1.09$ ).

**Seed weight.** The weight of 1000 cleaned and dried seeds of *D. lotus* was estimated to be 100.53 g. The seed weight does not include minor covering structures.

**Fruit size and weight.** There were significant differences among the shapes and sizes of fruits (Fig. 7).

*Adiantum capillus-veneris* L., *Phyllitis scolopendrium* (L.) Newman., *Dryopteris filix-mas* (L.) Schott., and *Acer campestre* L. were also found with the association of *D. lotus*. It was also found that *D. lotus* has associations with *Quercus castaneifolia* C.A. Mey., *Parrotia persica* C.A. Mey., *Acer velutinum* Boiss., *A. campestre* L., *Carpinus betulus* L., *C. orientalis* Mill., and *Potentilla reptans* L. in mixed forests of Lankaran district.



**Figure 7.** Variability of fruit size in the population of *Diospyros lotus* in Azerbaijan.

Each fruit contained between 1-10 seeds. The color of the fruits of *D. lotus* varies from yellow to blackish-brown. The average weight of 100 unripe fruits is 144.00 g. Variability of fruit size in the population was calculated as ranging from 2.2 cm to 0.97 cm.

**Distribution of study species in natural ecosystem.** *D. lotus* forms forests or grows as admixture in mixed forests. Forests with dateplum are adapted to humid, rich in humus and well-moistened soil [Mehdiyeva et al., 2016]. *D. lotus* is mainly found in mixed forest ecosystems in the studied areas. The predominant species in the forest of Qakh district were identified as *Pterocarya pterocarpa* Kunth ex I. Iljinsk., *Corylus colurna* L., and *Carpinus betulus* L. In addition, *Asplenium adiantum-nigrum* L., *A. trichomanes* L.,

## CONCLUSION

In this research work, we studied the morphological characteristics of the seeds of this species based on their size. Given that the morphological characteristics of the seed can significantly affect its germination, in this regard, our future research will focus on the study of germination ability. In other words, our future research is planned to focus on the relationship between the morphological characteristics of the seed of the *D. lotus* species and its ability to germinate.

## ACKNOWLEDGEMENT

Seed samples were collected in the frame of the joint projects (Global Tree Seed Bank Programme, 2016-2019; Threatened Biodiversity Hotspots Programme,

2020-2023) with the Millennium Seed Bank Partnership, Royal Botanic Gardens Kew. Seeds from Lankaran district were collected by Dr. H. Safarov in 2016, and by Dr. N. Mehdiyeva, Ph.D K. Asadova, Ph.D student N. Mursel in 2020.

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### Azərbaycanda *Diospyros lotus* (Ebenaceae) nadir bitki növünün toxumlarının morfoloji xüsusiyyətləri

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Bitkilər qidanın əsas mənbələrindən biri hesab edilir, eyni zamanda insan sağlamlığını qorumaq üçün bioaktiv birləşmələr ilə də zəngindir. *Diospyros lotus* L. qida kimi istifadə olunan meyvələri və qiymətli oduncağı ilə iqtisadi cəhətdən əhəmiyyətli bir növdür. Bitkinin yarpaqları, meyvələri və toxumları xalq təbabətində istifadə olunur. Tədqiqatın əsas məqsədi 2016, 2018

və 2020-ci illərdə Azərbaycanın şimal-qərbində və cənub-şərqində təbii yayılma arealında toplanan *D. lotus* növünün toxumlarının morfoloji xüsusiyyətlərinin öyrənilməsi olmuşdur. Toxumun tədqiqi üçün onun morfoloji xüsusiyyət kimi beş əlamətini qeyd etmişik: toxum uzunluğu, toxum eni, toxum sahəsi, toxum minimum eni və toxum perimetri. Nəticələr göstərdi ki, tədqiq olunan toxumların böyük faizi toxumun uzunluğu, eni və sahə nisbətləri 9.26-11.65 x 5.05-6.44 x 42.00-63.81 mm olan orta ölçüyə malikdir. Meyvələrinin ölçüsünün dəyişkənliyi isə 2.2 sm-dən 0.97 sm-ə qədər müəyyən edilmişdir. Bundan əlavə, *D. lotus*, təbii yaşayış mühiti daxilində ölkədə nəslə kəsilməkdə olan bir növdür və Azərbaycanın Qırmızı Kitabına daxil edilmişdir. Bu növün populyasiyasının həm regional, həm də dünya miqyasında təbii yayılma sahəsindəki azalmasını nəzərə alaraq, bu cür tədqiqatların aparılması vacibdir. Toxumların morfoloji dəyişkənliyi və fərqliliyi onların cücrəmə qabiliyyətinə də birbaşa təsir göstərə bilər ki, bu da növün qorunması üçün çox vacibdir. Aparılmış tədqiqat taksonomik əlaqələrin təhlili, filogeniyasını və statusunu dəqiqləşdirmək üçün əhəmiyyətli məlumatlar verə bilər.

**Açar sözlər:** adi xurma, meyvə çəkisi, Lənkəran, Qax, toxum forması, yabanı növ

### Морфологические характеристики семян исчезающего вида *Diospyros lotus* (Ebenaceae) в Азербайджане

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Растения являются одним из основных источников пищи, в которую также входят биоактивные соединения для поддержания здоровья человека. *Diospyros lotus* L. – экономически важный вид, обладающий съедобными плодами и ценной древесиной. Листья, плоды и семена растения используются в народной медицине. Целью исследования было



изучение морфологических характеристик семян и массы семян *D. lotus*, собранных с естественных ареалов этого вида на северо-западе и юго-востоке Азербайджана в 2016, 2018 и 2020 годах. Мы выбрали пять морфологических характеристик: длина семян, ширина семян, площадь семян, минимальный размер и периметр семян. Результаты показали, что большинство исследованных семян имеют средний размер со следующими соотношениями длины, ширины и площади: 9.26-11.65 x 5.05-6.44 x 42.00-63.81 мм. Изменчивость размеров плодов в популяции составила от 2.2 до 0.97 см. Более того, *D. lotus* является исчезающим видом в стране в пределах своей естественной среды обитания и занесен в Красную книгу Азербайджана. Учитывая текущее сокраще-

ние популяции этого вида в ареале естественного распространения как в региональном, так и в глобальном масштабе, проведение таких исследований является важным. Морфологическая изменчивость и различия семян также могут напрямую влиять на их способность к прорастанию, что очень важно для сохранения вида. Текущие исследования также могут быть полезны для анализа таксономических отношений, которые могут предоставить некоторые важные данные для понимания филогении и статуса изучаемых таксонов.

**Ключевые слова:** *хурма обыкновенная, форма семян, Ленкорань, Ках, масса плодов, форма плода, дикорастущий вид*