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Variability of morphometric traits of seeds of different genotypes of *Lycium* spp.

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Purpose. The objective of this study was to evaluate the morphological parameters of *Lycium* spp. seeds from the collections in M. M. Hryshko National Botanical Garden (NBS) NAS of Ukraine. **Methods.** Cultivars and varieties of three *Lycium* species (*Lycium barbarum*, *L. chinense*, *L. truncatum*) were studied in the period from 2016 till 2019. The following morphometric measurments were conducted: seeds weight, seeds length, seeds width and index of seeds shape. Basic statistical analyses were performed using PAST 2.17. Hierarchical cluster analyses of similarity between genotypes were computed on the basis of the Bray-Curtis similarity index. Correlation between traits was determined using the Pearson correlation coefficient. **Results.** Cultivars and varieties of different species of *Lycium* varied in weight, shape, and size of seeds. Seed weight varied from 0.54 to 3.54 mg, seed length from 1.90 to 3.06 mm, seed width from 1.43 to 2.53 mm. The shape indexes of seeds were found ranging from 0.73 to 0.80. The analysis of coefficient of variation showed the difference of variability in morphometric characteristics between some *Lycium* spp. cultivars and varieties. The most variable features: seeds weight (8.51–28.22%) and seeds length (5.07–24.81%) are important parameters for selection. The use of cluster analysis made it possible to establish the similarity between the species of the studied *Lycium* species. **Conclusions.** Diagnostic signs by seed morphometry for differentiation of *Lycium* species were revealed. The analysis of coefficient of variation showed the difference of variability in morphometric characteristics between some *Lycium* cultivars and varieties. The most variable characteristics of the studied genotypes were seed weight and length, which are important parameters for selection because they determine the pulp content and number of seeds, as well as the ratio of these parameters between them. It is through variability that promising varieties with low seed weight and length can be selected, Due to securing them later vegetatively.

Keywords: *goji berry; cultivars; varieties; seeds; parameters; cluster hierarchical analysis.*

Introduction

For the successful cultivation of neglected and underutilized species and the production of new valuable varieties, a comprehensive study of their plant morphology and anatomy is necessary [1–6]. It is especially important to study the biological characteristics of seeds, since they characterize the most successful varieties, especially those propagating by seed.

The morphological characteristics of the seeds of different plant species serve as taxonomical markings and also in deducing phylogenetic relationships [7] that would be a great help in academic as well as in applied ventures [8, 9].

The genus *Lycium* L. (Solanaceae Juss.) includes about 92 (97) species, of which 35 species are used as food and medicinal [10–12]. The two most common species are *L. barbarum* L. and *L. chinense* Mill., which have been used in Chinese medicine for over 2000 years [12, 13] because of content of valuable bioactive substances [14–18] that have many pharmacological effects, namely anti-cancer, anti-hyperglycemic, antioxidant, anti-inflammatory, and anti-aging properties [19–23]. Not only fruits, but also other plant parts, especially leaves contain valuable biologically active substances [24–26].

Lycium fruits are used to prepare juices, wine, canned food, used in soups, as porridge with rice, and added to various types of meat and vegetable dishes [12–18].

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The unique biochemical characteristics of *Lycium* are well documented. However, information about the morphological variability of *Lycium* seeds is insufficient. It is important to study the genetic variability of seeds for improving selected characteristics in the future.

The purpose of this study was to determine the variability of morphological characteristics of *Lycium* spp. seeds. The obtained results will help to select promising genotypes for further breeding work.

Material and methods

Collection of plant material

Plants growing in M. M. Hryshko National Botanical Garden of NAS of Ukraine (Kyiv) from seeds or cuttings obtained from China, France, Slovak Republic and other Botanical Gardens of Ukraine. The research was conducted during 2016–2019. The following genotypes of the three *Lycium* species were studied in this work: *L. barbarum* (var. LB01, LB02 and LB03); *L. chinense* (var. LC01, LC02, LC03, LC04, LC05, and cv. Amber Sweet, Big Lifeberry, Delikat, Q1, Sweet Lifeberry, Tybet); *Lycium truncatum* (var. LT01 and cv. Super Sweet, Korean Big, N1 Lifeberry, New Big, Princess Tao). The ripened fruits were harvested in the maturity stage (August).

Morphometric analysis

Immediately after the harvest, 30 fruits of each genotype were taken and 30 seeds were randomly selected. The following morphometric parameters were measured: seeds weight (50 seeds), in g; seeds length, in mm; seeds width, in mm. Seeds weight was measured by using a digital balance with a sensitivity of 0.01 g (PS6000/C/1). Linear dimensions of seeds as length and width were measured by using a digital calliper gauge with a sensitivity of 0.01 mm than shape index was calculated by using length/width ratio.

Statistical analysis

Basic statistical analyses – the minimal and maximal values of the traits, arithmetic means, and coefficient of variation (V %) were performed using PAST 2.17 (Norway, 2001). Results of the morphometric analysis were determined by mean ± standard deviation (SD) and statistical significance was estimated. Hierarchical cluster analyses of similarity between phenotypes were computed by the Bray-Curtis similarity index and were performed using PAST 2.17.

Results and discussions

For the first time since 2016 in Ukraine in the M. M. Hryshko National Botanical Garden (Department of Acclimatization of Fruit Plants) work on the collection of different species of *Lycium* L. has begun. Until this time, *Lycium* spp. was not studied in Ukraine at all. The collection consists of 45 genotypes (from seeds or cuttings) received from China, France, Slovakia and other botanical gardens in Ukraine, 9 of which were selected for cultivars.

There is limited information on morphometric parameters of *Lycium* seeds. Descriptions of species in the flora of countries indicate rough parameters of seed length and width indicators, which are usually 2–3 and 1.5–2.0 mm, or indicate only length. This does not reveal the morphometric parameters fully.

Cultivars and varieties of different species of *Lycium* plants varied in weight, shape, and size of seeds (Fig. 1).

The seeds color of the currently studied *Lycium* ranges from pale yellow, grayish yellow, and light brown.

The minimum and maximum values for the seeds weight, seeds length, seed width and shape index of seeds in the twenty-one cultivars and varieties are shown in Table 1.

Variation limits for seed length varied from 1.38 mm for LT01 (*Lycium truncatum*) to 2.20 mm for cv. Princess Tao (*L. truncatum*) (Table 1). The value of width varied within the interval from 1.01 mm (*L. barbarum* LB03) to 3.03 mm (*L. chinense* cv. Delikat). Seed weight ranged of 0.37 mg (*L. truncatum* cv. Princess Tao) to 4.43 mg (*L. chinense* LC03).

The average weight of the seed was determined in the range of 0.54 (*L. truncatum* cv. Princess Tao) to 3.54 (*L. chinense* LC03) mg, length of seed from 1.90 (*L. truncatum* LT01 and cv. New Big) to 3.06 (*L. chinense* cv. Delikat) mm, width of seed from 1.43 (*L. truncatum* LT01) to 2.53 (*L. chinense* LC03) mm (Fig. 2, 3).

Kazbekovna et al. [27] established a seed width range in *L. barbarum* from 2.50 to 3.0 mm and in *L. ruthenicum* from 1.5 to 2.0 mm. According to Zhang et al. [28], the seeds width of *L. ruthenicum* was also determined from 1.5 to 2.0 mm. The seeds width of *L. chinense* was determined to be between 2.5 and 3.0 mm and *L. shawii* between 1.5 and 2.0 mm [29].

The shape index (Fig. 4) of seeds which is ranged from 0.73 (*L. barbarum* LB04 and *L. chinense* LC01) to 0.80 (*L. chinense* LC03).

Fig. 1. Seeds of different *Lycium* species

Table 1

Variation limits of seeds of cultivars and varieties of *Lycium* spp.

Cultivars, varieties	Weight of 1000 seeds, g		Seeds length, mm		Seed width, mm		Shape index	
	min	max	min	max	min	max	min	max
Lycium barbarum								
LB01	1.81	2.87	2.19	2.97	1.58	2.46	0.64	1.02
LB02	2.30	3.37	1.90	3.65	1.45	2.75	0.64	0.93
LB03	0.75	2.14	2.00	3.03	1.01	2.57	0.39	1.12
LB04	1.33	2.04	2.36	3.04	1.69	2.63	0.58	0.88
Lycium chinense								
LC01	1.75	2.77	2.54	3.23	1.67	2.63	0.60	0.95
LC02	2.18	3.32	2.50	3.44	1.87	2.70	0.60	0.92
LC03	2.80	4.43	2.02	3.33	2.08	2.91	0.71	1.12
LC04	2.30	3.23	2.43	3.38	1.88	2.88	0.68	0.96
LC05	2.29	4.11	2.60	3.48	1.64	2.69	0.50	0.91
Amber Sweet	2.26	3.73	2.13	3.28	1.62	2.48	0.62	1.03
Big Lifeberry	1.86	2.99	2.46	3.06	1.64	2.50	0.60	0.96
Delikat	2.29	3.66	2.74	3.48	1.99	3.03	0.61	0.97
Q1	2.46	3.54	2.33	3.27	1.85	2.71	0.68	0.98
Sweet Lifeberry	1.49	2.37	2.32	3.31	1.77	2.76	0.62	0.99
Tybet	2.22	3.25	2.40	3.10	1.76	2.60	0.67	0.98
Lycium truncatum								
LT01	0.75	1.31	1.38	2.32	1.14	1.68	0.58	1.01
Super Sweet	0.72	1.42	1.69	2.46	1.12	1.96	0.53	1.05
Korean Big	0.52	1.10	1.45	2.38	1.09	1.80	0.47	1.00
N1 Lifeberry	0.69	1.02	1.57	2.40	1.19	1.78	0.63	0.93
New Big	0.45	0.78	1.59	2.26	1.15	1.81	0.57	0.98
Princess Tao	0.37	0.78	1.44	2.20	1.12	1.92	0.54	1.12

Note. min – minimum values; max – maximum values.

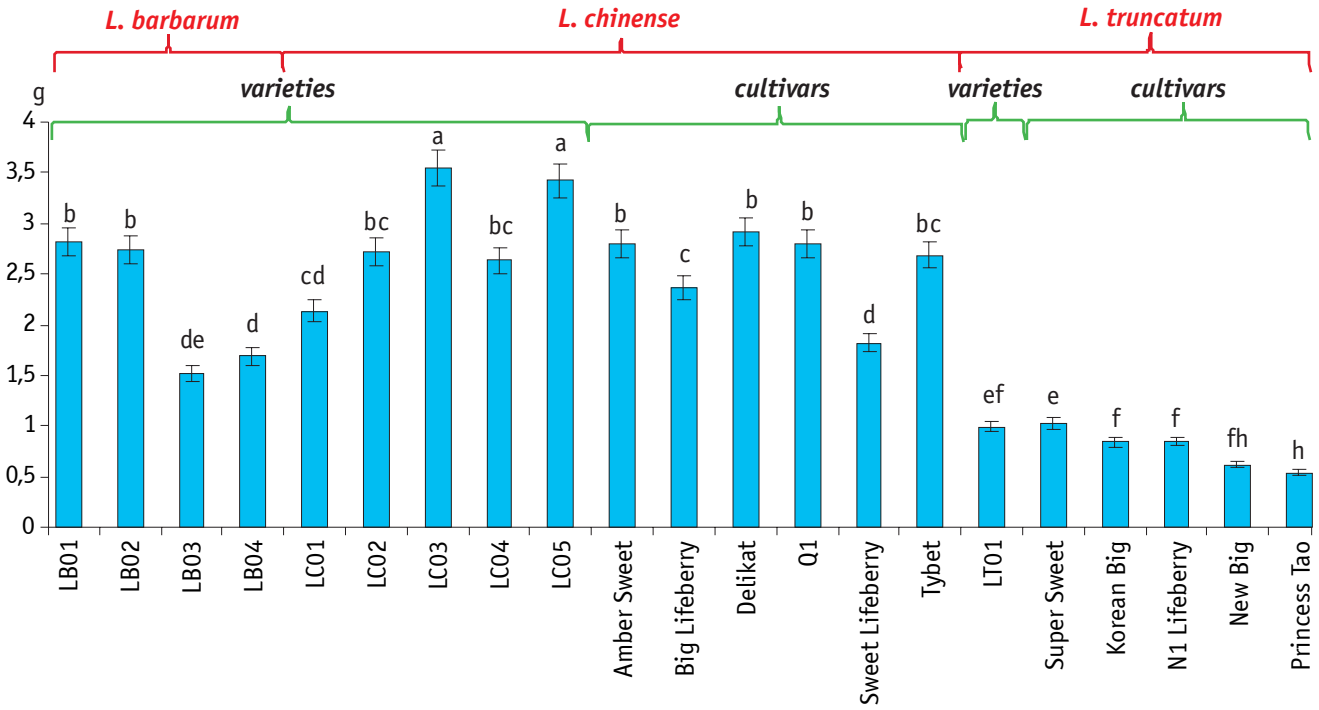

Fig. 2. Mean values for seed's weight of cultivars and varieties of *Lycium* spp.
[means in each column followed by different letters are not significantly different (P < 0.05)]

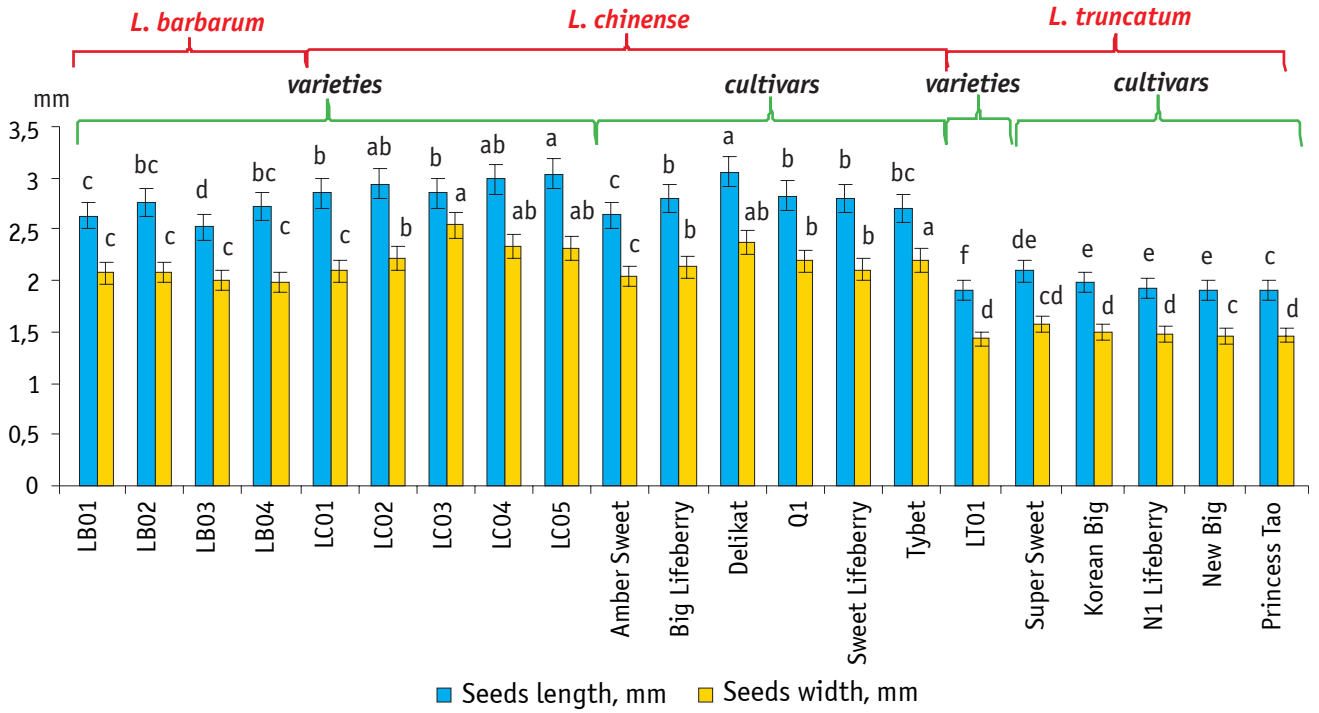

Fig. 3. Mean values for seed's length and width of cultivars and varieties of *Lycium* spp.
[means in each column followed by different letters are not significantly different (P < 0.05)]

The analysis of coefficient of variation showed the significant variability of morphological signs between cultivars and varieties (Fig. 5). The variation coefficients (%) ranged between 8.51 (*L. chinense* cv. Amber Sweet) and 28.22 (*L. truncatum* cv. Super Sweet) for seeds weight, between 5.07 (*L. chinense* cv. Big Lifeberry) and 24.81 (*L. barbarum* LB02) for seeds length, between 7.59 (*L. chinense* cv. Big Lifeberry) and 15.01 (*L. barbarum* LB02) for seeds width, between 8.51 (*L. chinense* cv. Amber Sweet) and 15.22 (*L. truncatum* cv. Super Sweet) for the shape index.

The most variable characteristics in the studied genotypes were seed weight and length,

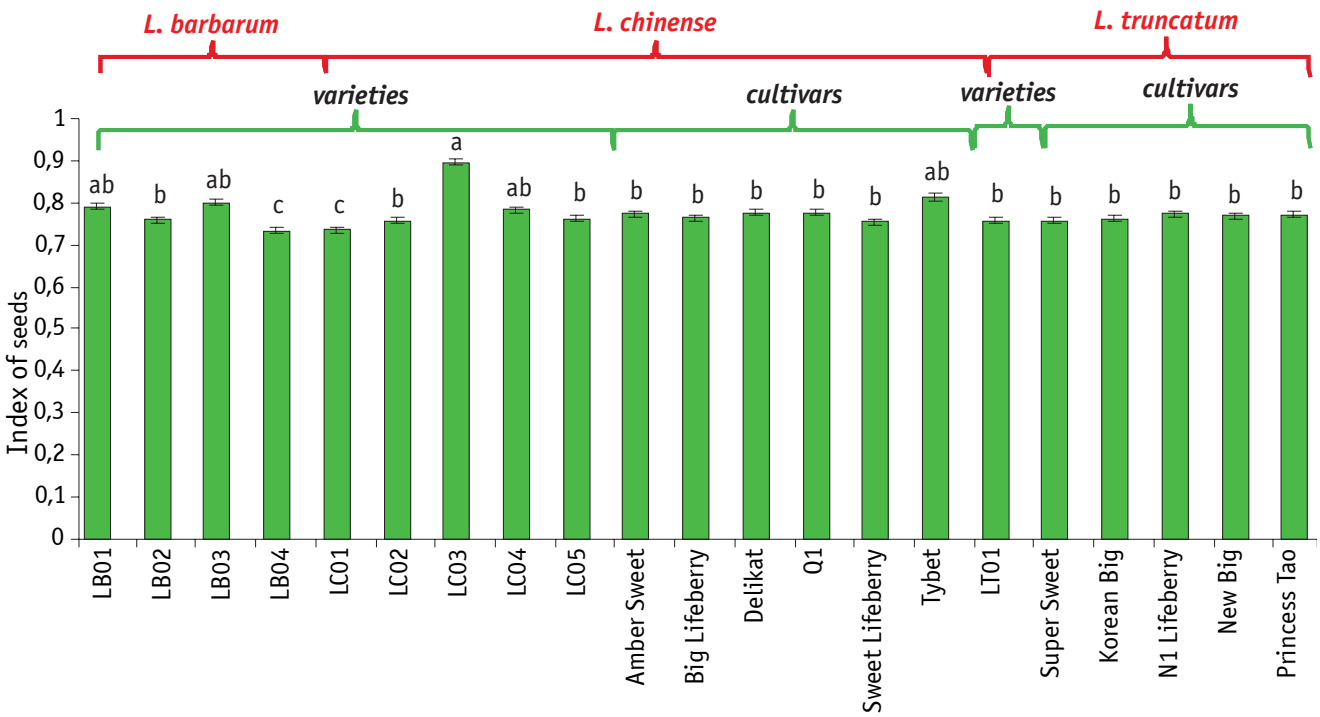

Figure 4. Comparison of shape index of seeds of cultivars and varieties of *Lycium* spp.
[means in each column followed by different letters are not significantly different (P < 0.05)]

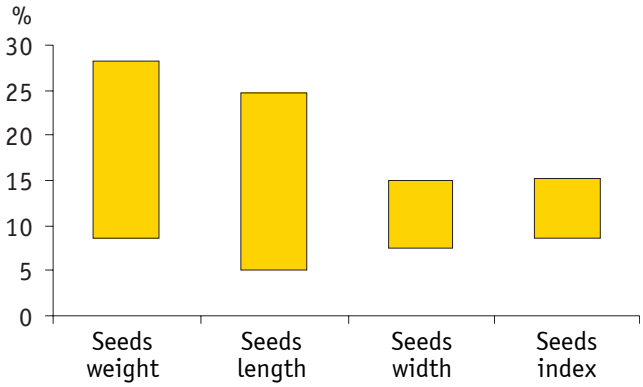

Fig. 5. Variability of morphological characters of *Lycium* spp. cultivars and varieties (%)

which are important parameters for selection. They determine the pulp content and number of seeds in the fruit and the ratio of these parameters to each other. The smaller the seed in weight and length, the greater the pulp content of the fruit. It is through variability that promising varieties with small weight and lengths can be selected, securing them afterwards, as in other cultivars, vegetatively.

Determination of the complex of relationships of morphological characteristics of seeds of cultivars and varieties of *Lycium* spp. showed a strong correlation between the main morphological features (Fig. 6).

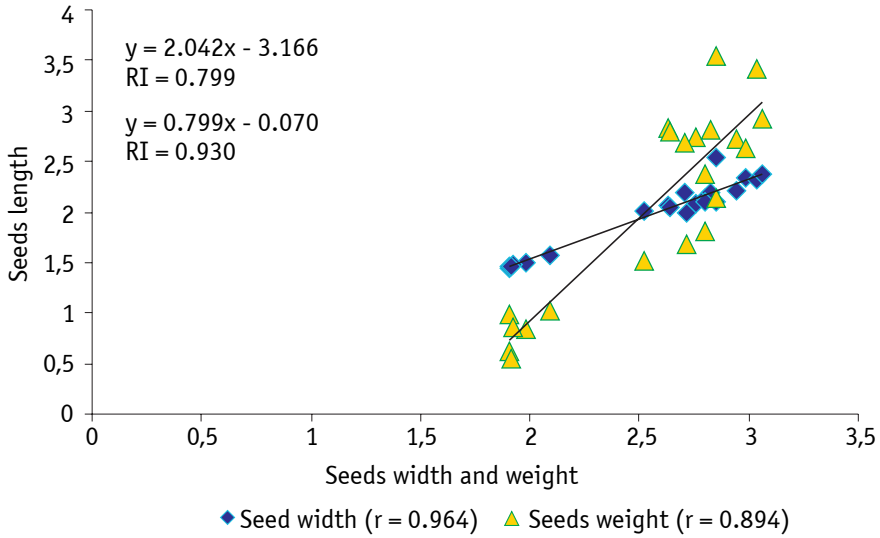

Fig. 6. Correlation of morphological parameters of seeds of cultivars and varieties of *Lycium* spp.

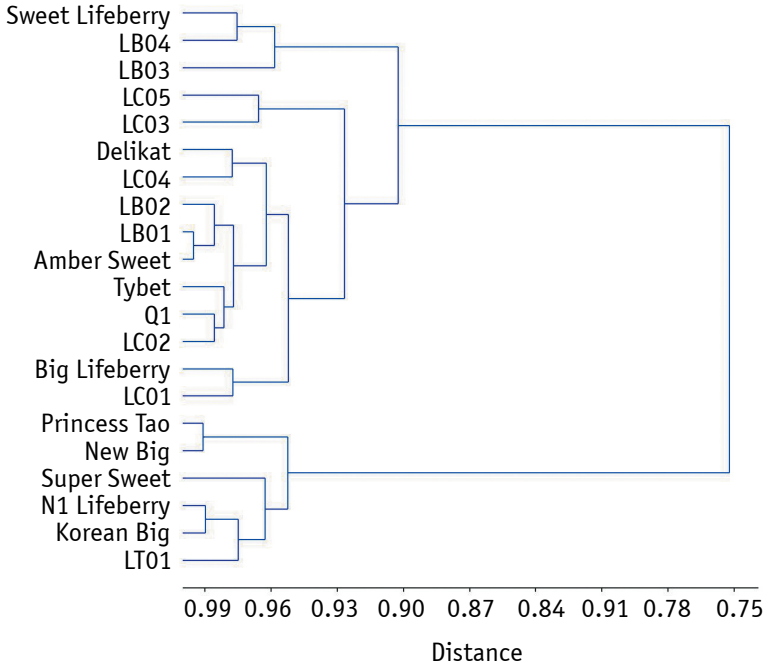

Fig. 7. Cluster dendrogram analyzed on four morphometrics parameters of seeds of 21 cultivars and varieties of *Lycium* spp.

Thus, the correlation coefficient between seed length and width was 0.964 and is reliable at all accuracy levels. The strength of the relationship between the length and seed weight was characterized by the coefficient of 0.894, and between the width and seed weight by 0.934.

Cluster analysis is very widely used to assess the genetic diversity of many plant species [1, 6, 30, 31].

The above data (Figure 2–5) confirms cluster analysis. In clustering, all studying parameters of seeds for 21 cultivars and varieties of the *Lycium* spp. were used, and the resulting clusters are shown in Figure 7.

Based on the data presented in Figure 7, we can say that cluster analysis divides the collection into two main clusters. The largest number of samples (15 cultivars and varieties) were included in Cluster I. Cluster II consisted of 5 cultivars and 1 varieties of *L. truncatum*, which were the most distant from all other samples of Cluster I, and differed from the others by the smallest morphometric characteristics of seeds. Cluster analysis demonstrates the integrated character of the variability in seeds of the studied species, cultivars and indicates possible ways to artificially improve the genetic material.

Varieties of *Lycium* with big seeds weight and size not be seen as a practical unusable. The seeds of different *Lycium* species contain rich biologically active substances, mainly fatty acids. Oil from these seeds can be used in the pharmaceutical and food industries [32–34].

Conclusions

Diagnostic signs by seed morphometry for differentiation of *Lycium* species were revealed. The analysis of coefficient of variation showed the difference of variability in morphometric characteristics between some *Lycium* cultivars and varieties. The use of cluster analysis allowed us to establish a clear limitation of *L. truncatum* on a complex of diagnostic characters. Seeds of *L. truncatum* differed from other plant species by the lowest morphometric indices. The most variable characteristics in the studied genotypes were seed weight and length, which are important parameters for selection. They determine the pulp content and number of seeds in the fruit and the ratio of these parameters to each other. The smaller the seed in weight and length, the more pulp the fruit contains. It is through variability that promising varieties with small weight and lengths can be selected, securing them afterwards, as in other cultivars, vegetatively.

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Журба М. Ю.[1*], Клименко С. В.[1], Шот І.[2] Мінливість морфометричних параметрів насіння різних генотипів *Lycium* spp. *Plant Varieties Studying and Protection*. 2021. Т. 17, № 1. С. 5–13. https://doi.org/10.21498/2518-1017.17.1.2021.228198

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Мета. Оцінити морфометричні показники насіння *Lycium* spp. колекції Національного ботанічного саду імені М. М. Гришка (НБС) НАН України. **Методи.** Упродовж 2016–2019 рр. досліджено 21 генотип (10 сортів і 11 форм) трьох видів (*Lycium barbarum*, *L. chinense*, *L. truncatum*). Установлено морфометричні показники насіння (маса, довжина, ширина та індекс форми). Статистичний аналіз виконували за допомогою PAST 2.17. Ієрархічний кластерний аналіз подібності генотипів здійснено за індексом подібності Брей-Кертіса. Наявність зв'язків між параметрами встановлювали за коефіцієнтом кореляції Пірсона. **Результати.** Сорти та форми різних видів рослин *Lycium* варіювали за масою, розміром та формою насіння. Морфометричні параметри насіння були такими: маса – від 0,54 до 3,54 мг, довжина – від 1,90 до 3,06 мм, ширина – від 1,43 до 2,53 мм. Величина індексу форми насіння становила від 0,73 до 0,80. Аналіз коефіцієнта варіації показав різну мінливість морфометричних характеристик сортів та форм різних видів *Lycium*. Наймінливішою є маса насіння (8,51–28,22%) та його довжина (5,07–24,81%), які є важливими параметрами для селекції. Використання кластерного аналізу дало змогу встановити генетичні зв'язки між сортами й формами *Lycium* та розподілити їх у два основних кластери. **Висновки.** Виявлено діагностичні ознаки морфометричних параметрів насіння для ідентифікації видів *Lycium*. Аналіз коефіцієнта варіації показав мінливість морфометричних характеристик між деякими сортами та формами *Lycium*. Наймінливішими параметрами досліджуваних генотипів були маса та довжина насіння. Останні є важливими для селекції, оскільки від них залежить уміст м'якушу та кількість насіння, а також співвідношення цих параметрів між ними. Завдяки мінливості можна дібрати перспективні сорти з невеликими масою та довжиною насіння, закріпивши їх потім вегетативно.

Ключові слова: *годжі; види; сорти; форми; насіння; морфометричні параметри; кластерний ієрархічний аналіз.*

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