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Variability of morphological parameters of *Diospyros lotus* L. flowers

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The morphological variability of the male and female flowers of 23 date plum (*Diospyros lotus* L.) genotypes found together at date plum collection in Arboretum Mlynany (Slovak Republic) was investigated. The collection consists of seed-propagated genotypes, which were established in 1970 with introduced seeds from different countries. Results showed that male and female flowers of *D. lotus* genotypes differed from each other in terms of shape and size. According to the morphological analysis, several features were found variable both within and between different plants. Moreover, significant differences in the colour of their corolla were detected. Female and male flowers of *D. lotus* genotypes were grouped according to the absolute parameters of flowers, and five (male flowers) and three (female flowers) clusters were identified. The degree of affinity between species was assessed using values of Euclidean distance. Using the principal component analysis, female and male flowers were separated into groups with similar morphological parameters. Our investigation has extended the understanding of the morphological features of *D. lotus* flowers as compared to the previously published data, which were limited only to a short description.

Keywords: date plum, flowers, morphometrical variability

Introduction

Discussing the negative impact of alien invasive species on natural biological diversity, we ask the primary question: what features of the introduced species contribute to its invasion? One of the hypotheses explaining the successful invasion of plants into the natural plant communities is one of high genetic variability of the initially introduced populations (Allard, 1965; Lavergne and Molofsky, 2007). The individual variation is a key determinant of the spread rate of species through landscapes (Sakai et al., 2001; Suda and Pyšek, 2010; Jongejans et al., 2011; Parker et al., 2013; Feulner et al., 2017). This hypothesis is supported by the example of a number of plant species including *Amaranthus* albus L., *Ribes aureum* Pursh, *Acer negundo* L., *Impatiens glandulifera* Royle,

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I. parviflora DC, *Bidens frondosa* L., *Conyza canadensis* L. In the present study, we test this explanation on the example of *Diospyros lotus* L., which is currently considered a potentially invasive species that invade natural plant communities of Southern Europe and Asia Minor.

D. lotus (Ebenaceae) is a deciduous tree, which grows naturally in China and other Asian countries (Yonemori et al., 2000; Bellini and Giordani, 2005). In cultural form, this species is spread in Korea, Pakistan, Afghanistan, Turkey, Albania, Spain, France, and Poland (Yonemori et al., 1998; Ayaz and Kadioglu, 1999; Ercisli and Akbulut, 2009).

D. lotus is a relict of the tertiary flora and one of the most ancient of modern deciduous moderately thermophilic species, which arose at the later stages of the evolution of the genus *Diospyros*. Under natural conditions, the height of this tree is up to 15 m (Ayaz and Kadioglu, 1999; Ercisli and Akbulut, 2009). It has been cultivated in several countries for its edible fruits and has also been used as a rootstock source for *D. kaki* L. (Onur, 1995; Ercisli and Akbulut, 2009).

D. lotus which is produced for its fruits has become invasive in Black Sea forests, adapting to the Mediterranean forest ecosystem, and damage to natural lands. This plant species has been acquired as invasive and today, it is devastating natural forest ecosystems and biological diversity (Necmi, 2011). *D. lotus* is been considered in Mediterranean Regions as one of the 20 transformers that are declared emerging invaders. This group of future major invaders, their "transformer behaviour" appears as early as the first establishment in primary biotopes and the further spreading of the species is then predictable (Brunel and Tison, 2005). There is evidence that *D. lotus* occurs on road embankments in Hyrcanian forests (Parsakhoo et al., 2009).

In Central Europe, the species has just begun naturalization. *Diospyros lotus* was found outside the Buda Arboretum of the Corvinus University of Budapest and also in many urban spaces in Hungary (Sütöri-Diószegi and Schmidt, 2010). However, in Switzerland, *Diospyros lotus* was included in the group of unsuccessful exotic plant species (Weber and Gut, 2004). The settlement of this species needs to be monitored, especially in connection with climate warming. This species may become the same "escaping from cultivation", as some ornamental plants (Pergl et al., 2016).

One of the real ways of detecting the adaptive potential of any species under introduction is by researching its variability. It is well known that the intraspecific diversity of the plant greatly increases during long-term breeding (Kohno and Kuznetsov, 2005) whereas in natural optimal conditions of growth, its variability is much less. The variability of features observed in species, which are grown up in different habitats, is caused by differences in their ecological conditions (Mamaev, 1972). Such data give the possibility to find out the adaptive mechanisms, which preserve the viability of species in certain environmental conditions (Mikovski et al., 2021).

To test the hypothesis of the high variability of successfully colonizing species, it is necessary to estimate the variability of their phenetic and genetic traits. We have previously described the degree of the variability of *Diospyros lotus* fruits (Grygorieva et al., 2009c). In this study, we estimate the variability of the morphometric parameters of the male and female flowers of this species in the introduction population of Slovakia.

Material and methodology

Locating trees and data collection

The studies were conducted in 2017 in the Arboretum Mlynany (Slovakia). The objects of the investigation were male (DLm-01–13) and female (DLf-01–10) plants of 23 *Diospyros lotus* genotypes grown from seeds, which have been brought from different habitats (South Korea, China, and Japan). The plants were planted at two years of age in 1970. They are well adapted to the climatic and soil conditions.

Morphometrical characters

13 male (staminate) and 10 female (pistillate) genotypes were investigated. From each tree, we sampled 30 flowers during the blossoming. Generally, we have analyzed 390 female and 300 male flowers. The morphological variability was defined by morphometric investigations of the following characteristics (Figure 1): length of corolla (CL), width of corolla (CW), mm; length of petals (PL), width of petals (PW), mm; number of petals (PN), pcs; length of sepals (SL), width of sepals (SW), mm; number of sepals (SN), pcs; amount of stamens of epipetalous whorl (NS1), amount of stamens of episepalous whorl (NS2), pcs; length of stamens of epipetalous whorl (LS1), length of stamens of episepalous whorl (LS2), mm: amount of staminodes (NS), pcs; length of staminodes (LS), mm;

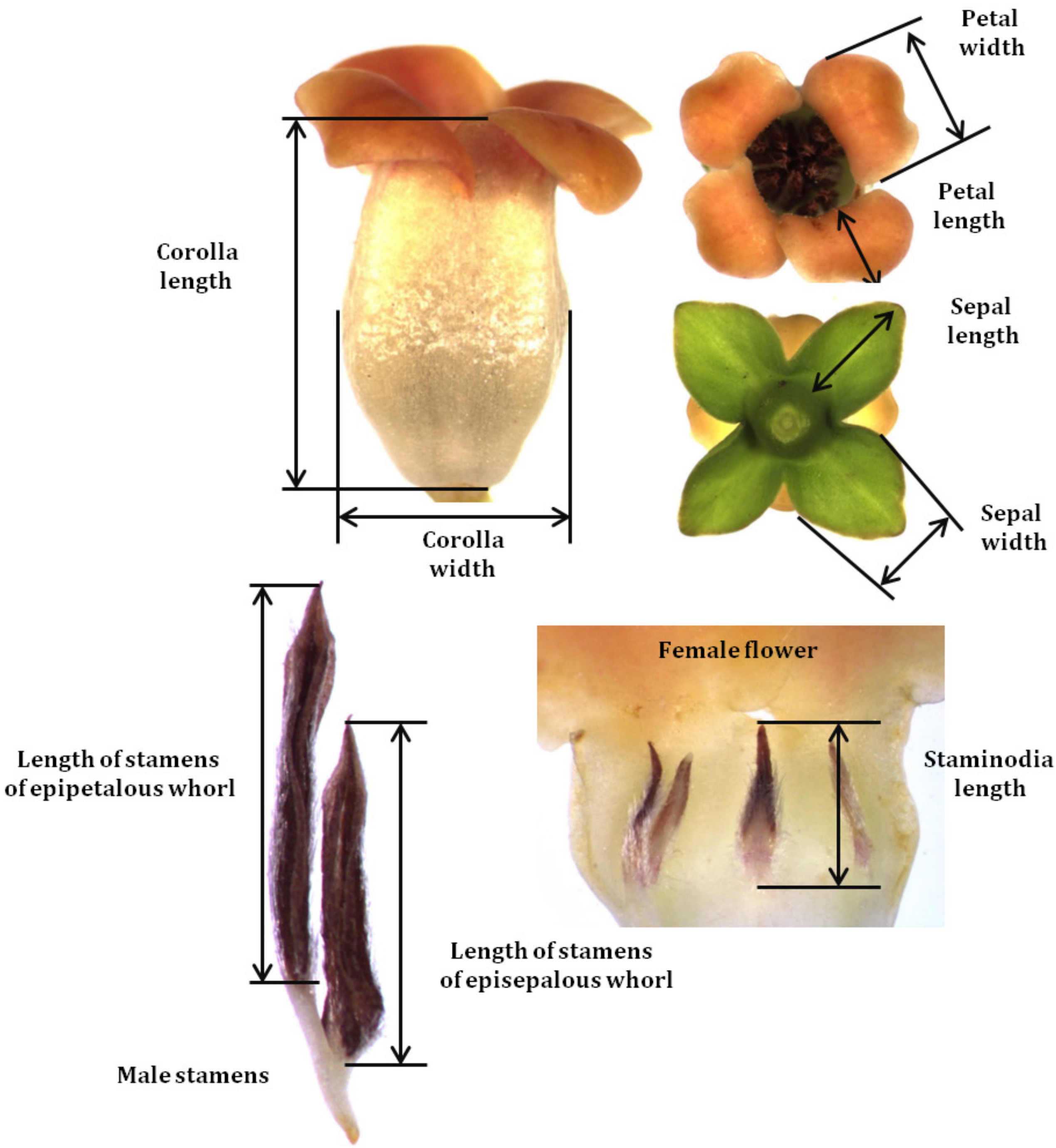

Figure 1 Flowers of *Diospyros lotus* L. showing the floral characteristics measured

shape index (CL/CW). The measurements were carried out on photo macrographs. The image analysis was carried out using the software AxioVs40 V 4.8.2.0.

Statistical analysis

Basic statistical analyses were performed using PAST 2.17 to calculate numerical characteristics such as sample size (n), range (minimum and maximum), mean value, standard deviation (SD), and coefficient of variation (CV) of a trait; hierarchical cluster analyses of similarity between genotypes were computed based on the Euclidean distance similarity index. The level of variability was determined by Stehlíková (1998). Principal component analysis (PCA) was performed to evaluate relationships among variables and any possible genotype groupings based on similar properties by using an XLSTAT procedure (XLSTAT 7.5, Addinsoft, USA).

Results and discussion

Population growth plants and climate change pose a serious threat to many ecosystems. Therefore, identifying new or rare species is becoming increasingly important. The geographical assignment of species is also of great relevance to broad biodiversity conservation projects. Morphometrics, the study of shapes of leaves, petals, and whole plants, has been applied to plants for many years and is of great importance to science, as it can help to distinguish between different species, to measure plant health, and even to model climate change (Cope et al., 2012).

The study of flower morphology is of particular interest because flowers are the most complex structures of the plant organism. In addition, the identification of morphometric parameters of flowers is necessary for the reliable detection of interpopulation changes (Rozov and Deineko, 2018). It is also very important studying from the interaction of a plant with a pollinator to plasticity and response to biotic and abiotic stresses (Jolles, 2015; Carleial et al., 2017; Sauquet et al., 2017; Spencer and Kim, 2018; Mikovski et al., 2021).

Descriptions of the morphology of the flowers of *D. lotus* are presented in Hiern (1873), Shu-Kang et al. (1996), and Sponberg (1977) works. But they are confined only by a brief description: female flowers subsessile, solitary on short, pubescent, bracteate pedicels 2–3 mm long; calyces green, 7–8 mm long, densely rufous-pubescent on the adaxial surface of the calyx tube, accrescent in fruit and sometimes persistent on the branchlets, the 4 or 5 lobes foliaceous, 6–7 mm long at anthesis; corolla reddish-brown, broadly urceolate, ca. 5 mm long, the lobes 2–3 mm long, more or less recurved; staminodes 8, curved over the surface of the ovary, pubescent with long, silvery hairs. Male flowers produced on the current year's growth, held ± nodding beneath the leaves. Staminate flowers are 6–7 mm. Long at anthesis, short-pedicellate, 3–5 together (or fewer through abortion) in rufous-pubescent, short-pedunculate, the bracts caducous; calyces green, finely pubescent, the 4 (or 5) deltoid lobes 1.5–2 mm long; corolla 4.5–6 mm long, white, ± campanulate and weakly 4- (5-) ribbed, the lobes pinkish or yellowish, recurved, ca. 2 mm long; stamens 16, rarely fewer, epipetalcus in 2 whorls, the largest stamens ca. 4 mm long; gynoecia abortive or rudimentary (Sponberg, 1977).

The investigation of *D. lotus* under introduction conditions shows evident polymorphism, which affects (nearly) all morphological features of this species (Grygorieva et al., 2009a, 2009b). We can find the varieties of all features which fluctuate in highly visible limits. In this case, not only the special features of the vegetative sphere of the plant are exposed to changeability, and also the constant features of generative organs (flowers), which are usually considered the most conservative.

Figure 2 The variability of size and shape of calyx
1 – male flowers (a – from one plant; b – from different studied plants); 2 – female flowers

Figure 3 The colour of the edges of sepals of female flowers

As a result of our investigation, it was ascertained that flowers of *D. lotus* are strongly differentiated in shape, size, and colour of the corolla of both the male and female flowers (Figure 1–5).

According to our observations, the sepals of male and also female flowers are quite different in shape. There is a highly variable form of sepals of the same plant (Figure 2). Female flowers have bigger sepals than male flowers. Sepals of female flowers unlike male flowers are much larger than the corolla. The inner side of the sepal of both female and male flowers is always extremely densely pubescent. The hairs are colourless and pointed.

Usually, sepals are green and have different shapes. During the investigation, we found one plant, which had different coloured edges of sepals (Figure 3). Sepals of female flowers are longer and wider than the sepals of male flowers.

The corolla of female flowers is longer and wider than the corolla of male flowers. The corolla length of female and male flowers in our analyses was determined

Figure 5 The variability of colour of petals of corolla
1 – female flowers; 2 – male flowers

in the range from 3.29 to 10.02 mm and from 2.66 to 7.15 mm, respectively. Petals of female and male flowers are thick, and waxy, 2.37–7.46 mm and 1.07–4.92 mm length, 2.16–7.40 mm and 1.89–4.92 mm width, respectively (Table 1). They are usually 4, but there are flowers with 2 (male flowers) and 7 (female flowers)

Figure 4 The number of petals of corolla
1 – female flowers; 2 – male flowers

Table 1 Morphological characteristics of features of flowers of *Diospyros lotus* L.

Morphological parameters	min	max	mean	CV%	min	max	mean	CV%
		male flowers				female flowers		
Length of corolla (mm)	2.66	7.15	4.95	13.76	3.29	10.02	6.40	18.52
Width of corolla (mm)	2.10	6.77	4.53	11.93	4.0	8.09	5.28	12.48
Petals length (mm)	1.07	4.92	3.06	21.64	2.37	7.46	4.76	16.39
Petals width (mm)	1.89	4.92	3.40	13.79	2.16	7.40	4.48	23.61
Petals number	2.0	6.0	4.14	12.08	3.0	7.0	4.14	12.36
Sepals length (mm)	1.03	5.10	2.27	29.53	2.57	8.52	5.96	22.90
Sepals width (mm)	1.49	4.90	2.38	22.54	1.94	8.34	5.42	21.07
Number of sepals	3.0	6.0	4.13	11.78	3.0	6.0	4.11	10.61
Amount stamens of epipetalous whorl	7.0	9.0	7.97	4.21	–	–	–	–
Amount stamens of episepalous whorl	7.0	9.0	7.96	4.16	–	–	–	–
Length of stamens of the epipetalous whorl (mm)	2.12	4.72	3.45	8.92	–	–	–	–
Length of stamens of the episepalous whorl (mm)	2.09	3.84	2.95	11.69	–	–	–	–
Staminodes amount	–	–	–	–	7.0	10.0	7.99	4.53
Staminodes length	–	–	–	–	1.48	3.68	2.29	17.20

min, max – minimal and maximal measured values; mean – arithmetic mean; CV – coefficient of variation, %

petals (Figure 4). The length and width of *Diospyros lotus* of flowers of the present study were in the range from 2.57 to 8.52 mm and from 1.94 to 8.34 mm (female flowers), from 1.03 to 5.10 mm and from 1.49 to 4.90 mm (male flowers), respectively.

The stamens are often different in size. The length of stamens of the epipetalous whorl is from 2.12 to 4.72 mm and the episepalous whorl is from 2.09 to 3.84 mm (Table 1). Stamens of female flowers are reduced (staminodes). They are from 7 to 10. The staminodes are with short staminal threads, which are attached to the base of the corolla just two per one petal 1.48 to 3.68 mm long.

According to the morphological analysis, a lot of features vary both within a single plant (Figures 6 and 7) and between different plants (Table 1). This is confirmed by other studies on the same plant species (Armbruster, 1991; Williams and Conner, 2001; Ishii and Morinaga, 2005; Zhao et al., 2010), which allows us to estimate the individual and interpopulation variability of the species (Arteaga et al., 2015).

In conducting of statistical data the coefficients of variability of each morphological feature of generative organs of *D. lotus* were calculated. As a result, the fact of the existence of a certain degree of variability was confirmed.

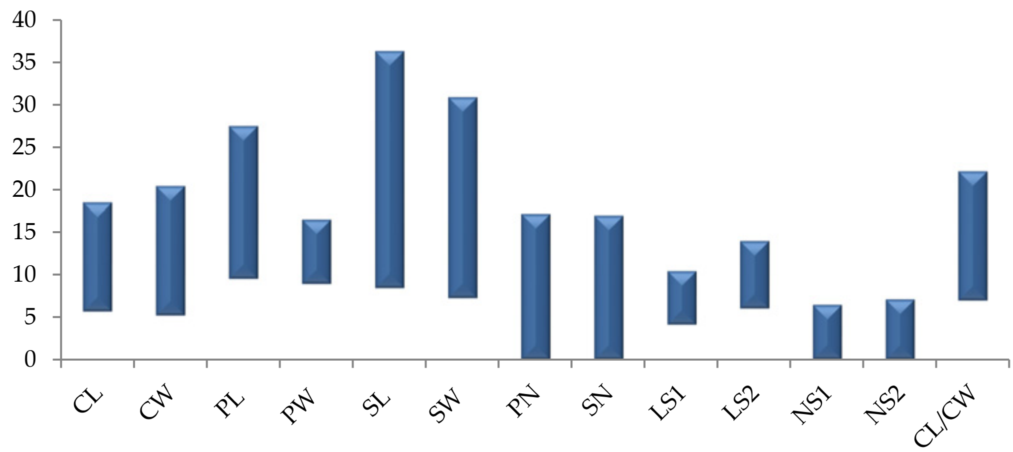

Figure 6 Level of the variability of morphometric parameters of male flowers of *Diospyros lotus* L. , %

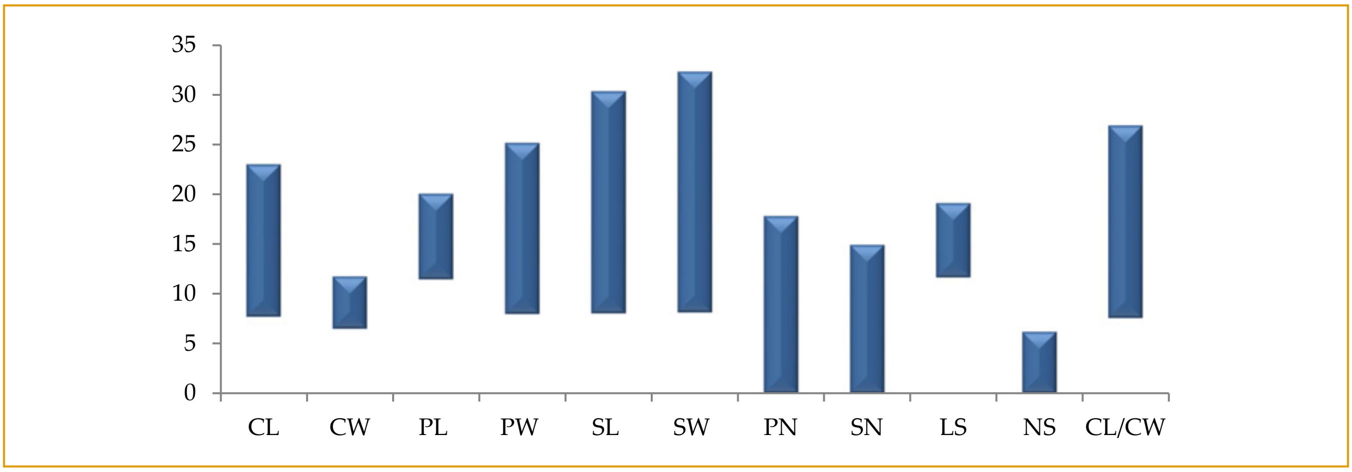

Figure 7 Level of the variability of morphometric parameters of female flowers of *Diospyros lotus* L., %

The range of variation of morphological features of male flowers is 4.11–36.31% (Figure 6). The high level of variability is determined for the length and width of sepals, length of petals with coefficients of variation 36.31, 30.88, and 27.45%, respectively. The least variable parameters are the length of stamens of epipetalous whorl and episepalous whorl (6.31 and 7.96%), the number of stamens of epipetalous whorl and episepalous whorl (6.45 and 7.10%), respectively. The other morphological features have an average degree of variability. Such a result is not surprising, because it is well known that the internal organs of the flower such as pistils and stamens are the least variable, so the classification of plant life is based on these features.

The range of variation of morphological features of female flowers is 8.0–32.38% (Figure 7). A high level of variability was found for the width and length of the sepals, shape index of the corolla, width of petals, and length of corolla width value of the coefficient of variation 32.38, 30.38, 26.94, 25.18 and 23.02%, respectively. These data are consistent with the results of other researchers who have compared the variability of perianth and internal flower structures (Herrera et al., 2008).

An acceptable solution for principal component analysis was reached when two dimensions of the model were found to be significant and explained 54.68% of the total variance of the original variables set (Table 2). The first component (PC1), accounting

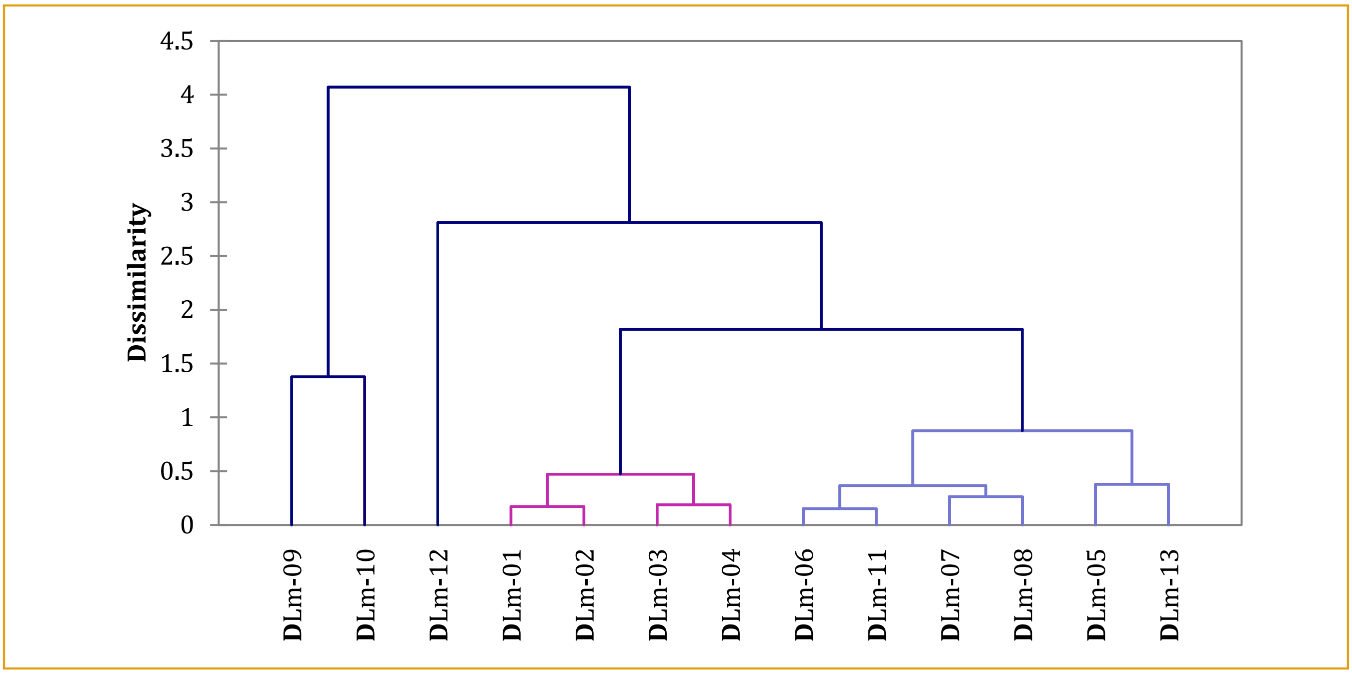

Figure 8 Cluster dendrogram based on morphometrics parameters of *Diospyros lotus* male flowers genotypes

Table 2 Eigenvalues, the proportion of total variability, and correlation between the original variables and the first four principal components for *Diospyros lotus* male flowers genotypes

Variable	Male flowers		Female flowers	
	PC1	PC2	PC1	PC2
CL	0.92	-0.15	0.77	-0.47
CW	0.68	-0.16	-0.17	-0.06
PL	-0.35	-0.12	-0.42	-0.44
PW	-0.19	0.41	0.88	0.15
SL	0.76	0.24	0.65	0.55
SW	0.63	0.57	0.74	0.12
PN	0.56	-0.38	-0.10	0.06
SN	0.55	-0.38	-0.39	-0.72
LS1	-0.53	-0.60	–	–
LS2	-0.75	-0.34	–	–
LS	–	–	-0.47	0.68
NS1	-0.45	0.76	–	–
NS2	-0.02	0.86	–	–
NS	–	–	-0.07	0.74
CL/CW	0.43	-0.07	0.81	-0.40
Eigenvalue	4.35	2.75	3.62	2.51
Variability (%)	33.47	21.21	32.93	22.82
Cumulative (%)	33.47	54.68	32.93	55.74

for 33.47% of the total variance, is dominated by male flower characters, namely length of corolla (CL) and length of sepals (SL). In the second component (PC2) dominated the number of stamens of the epipetalous whorl (NS1), the amount of stamens of the episepalous whorl (NS2) were the main contributors and explained 21.22% of the total variance.

The PCA used in our work in researching female flower genotypes showed that more than 73.74% of the variability observed was explained by the two components (Table 2). Positive values for PC1, accounting for 32.93% of the total variance, correspond to the genotypes with a higher length of corolla (CL), petals width (PW), and shape index (CL/CW). In the second component (PC2) dominated of the length of staminodes (LS) and amount of staminodes (NS) were the main contributors and explained 22.82% of the total variance.

The cluster and principal component analysis on the morphological characters have been carried out earlier for studying the variability of some other plant species (Vijayan et al., 2006; Al-Ruqaie et al., 2016; Krishnapillai and Wijeratnam, 2016; Yilmaz et al., 2017) and he may also be used as useful tools for screening the accessions (Bellini et al., 2003; Ercisli, 2004; Yildirim et al., 2010; Horčinová Sedláčková et al., 2021) and for effective management of genetic resources that is the first step toward any domestication process (Metougui et al., 2017).

By applying cluster analysis to variables retained by the PCA dendrogram in Figure 8, five groups of genotypes of male flowers were differentiated at a dissimilarity level of 1.0. Cluster I consisted of four genotypes (DLm-01, DLm-02, DLm-03, DLm-04). Cluster II included the largest number of genotypes (DLm-05, DLm-06, DLm-07, DLm-08, DLm-11, and DLm-13). Each cluster III, IV, and V included only one genotype.

The morphological similarity among 10 genotypes based on morphometrical characteristics of female flowers was examined by cluster analysis too (Figure 9). Dendrogram had shown three main clusters. Three of the 10 genotypes were included in Cluster I, 3 genotypes in Cluster II, and 4 genotypes in Cluster III.

Principal component and cluster analysis discriminated the sampled genotypes into five clusters (Figure 10) using the first two principal components and accounted for about 54.68% of the total variability among the *Diospyros lotus* genotypes, based on male flower traits, respectively.

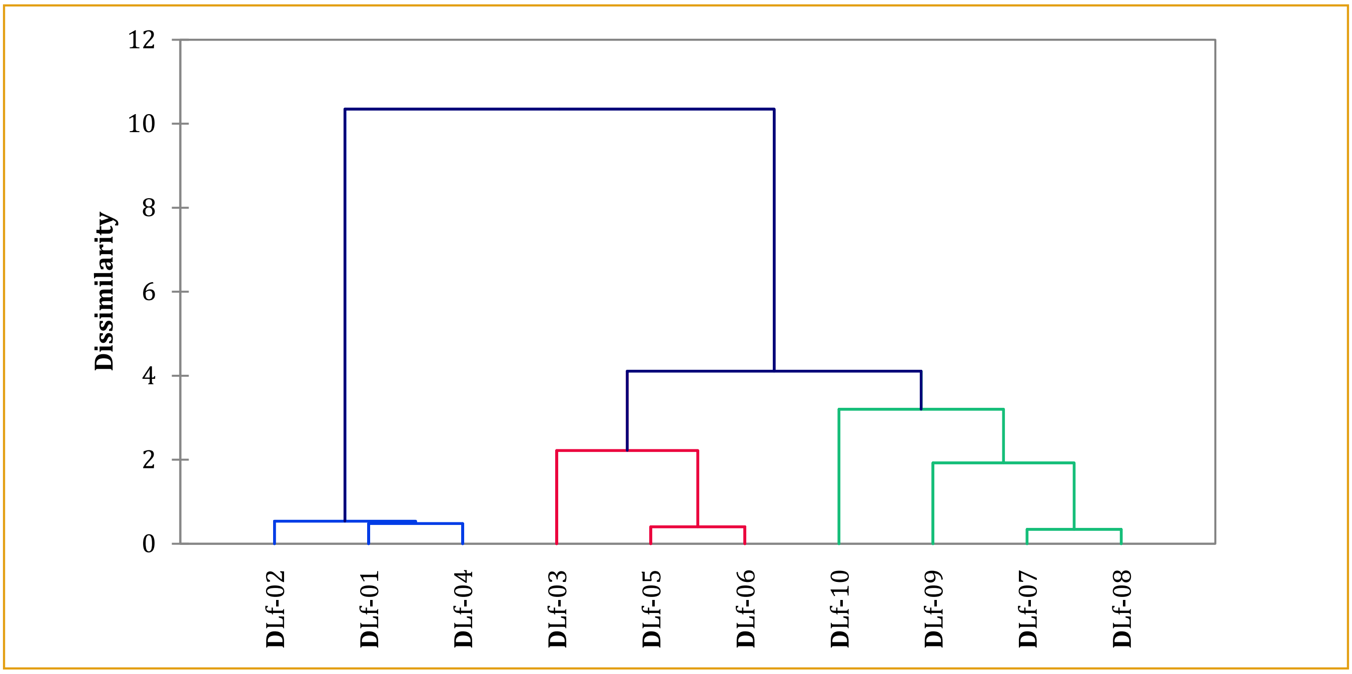

Figure 9 Cluster dendrogram based on morphometrics parameters of *Diospyros lotus* female flowers genotypes

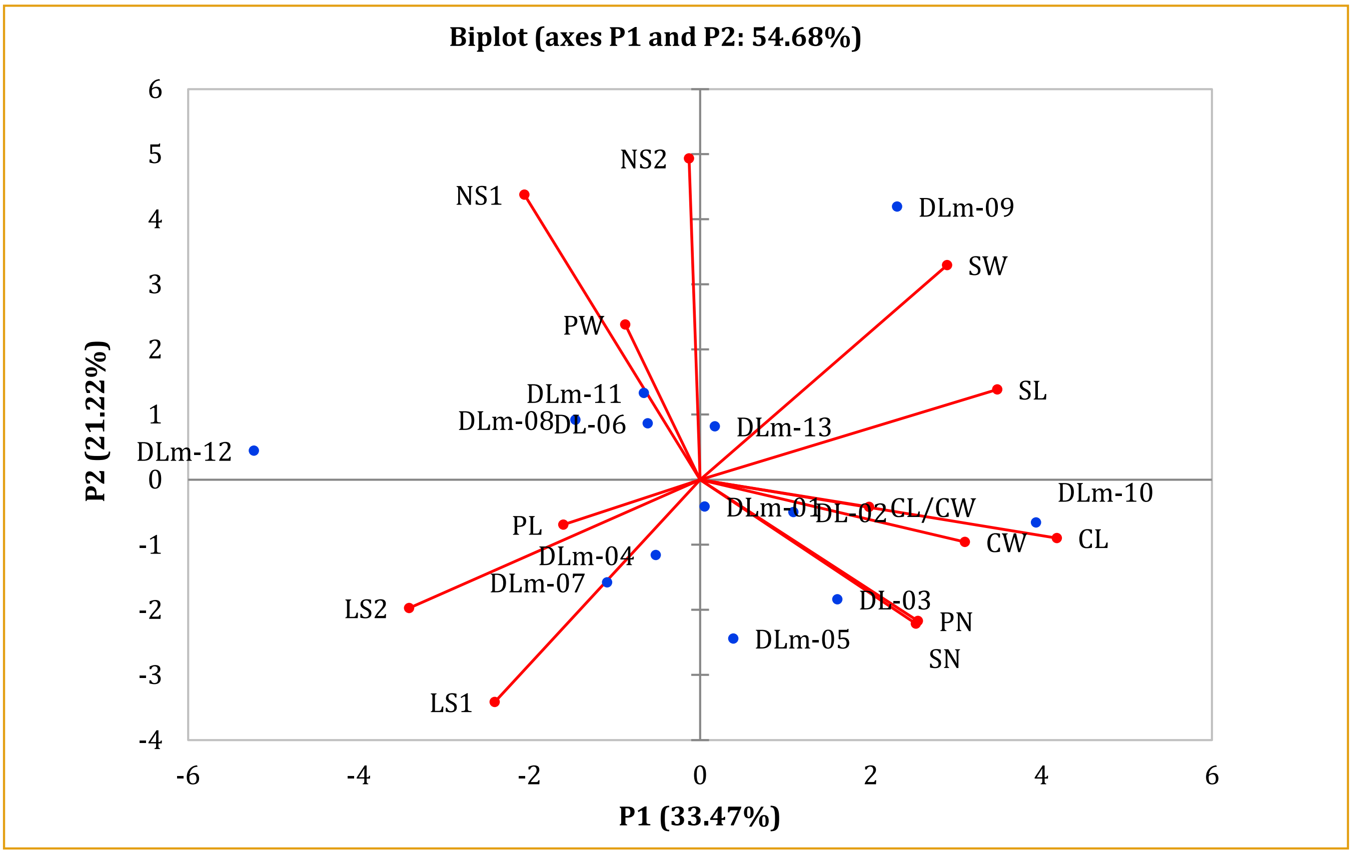

Figure 10 Biplot based on principal components analysis (PCA) for male flowers characteristics in *Diospyros lotus* L. genotypes

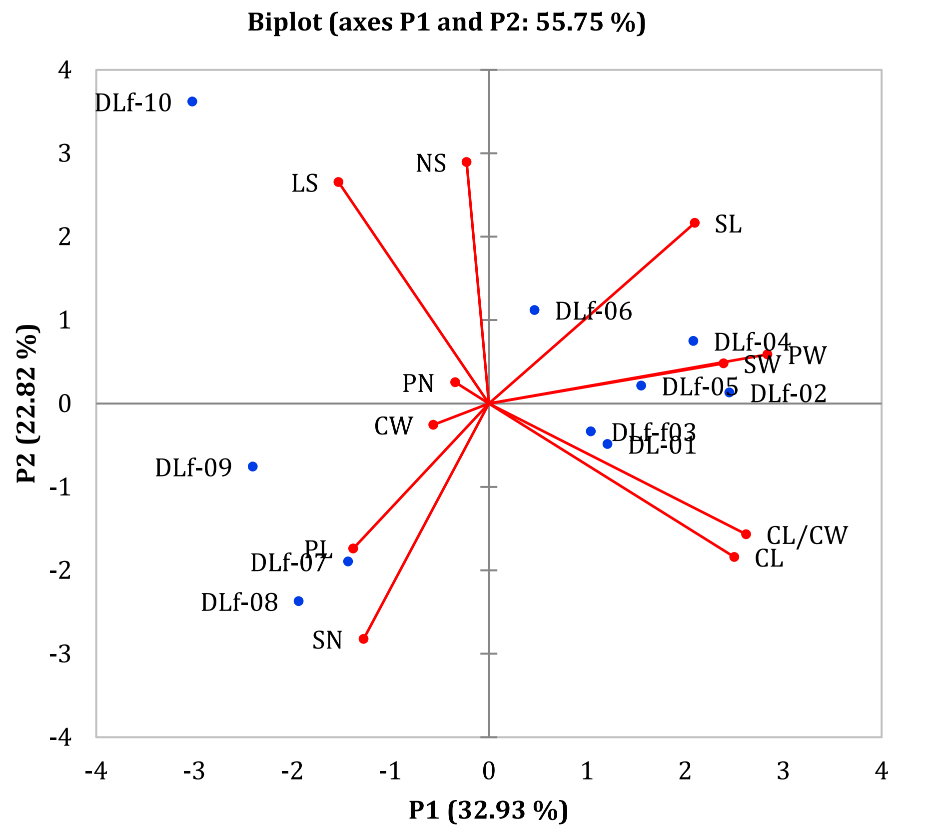

Figure 11 Biplot based on principal components analysis (PCA) for female flowers characteristics in *Diospyros lotus* L. genotypes

Regarding the flowers of female plants principal component and cluster analysis discriminated the sampled genotypes in three clusters (Figure 11) using the first two principal components and accounted for about 55.75% of the total variability among the *Diospyros lotus* genotypes, based on female flowers traits, respectively.

Conclusions

As we mentioned earlier, only a short description of the flower morphology of *Diospyros lotus* has already been studied. With these data, we contribute to the knowledge about this species. As a result of our research was ascertained that flowers of *Diospyros lotus* are very different in shape, size, and colour of both male and female flowers. The coefficient of variation of morphological features of female flowers ranges from 8.0 to 32.38% and male flowers – from 4.11 to 36.31%. The fluctuations of the values of investigated features characterize the plasticity of this species; which can be considered as some kind of adaptive reaction to changing ecological conditions. The high values of the coefficient of variability among the features of the generative sphere of *Diospyros lotus* can be explained by optimal conditions for this species. In the future, obtained data on the variability can be used as a theoretical and practical base for a whole series of problems in general biology. Thus, the introduction population of *Diospyros lotus* in the Mlynany Arboretum (Slovakia) is represented by numerous varieties. Its phenetic and genetic variability is quite large. On the one hand, this determines the stability of the species in the culture, and on the other, it can, because of the cross-pollination, contribute to the emergence of new genotypes that are more resistant to the soil and climatic conditions of Central Europe. We do not exclude the possibility of further expansion of the invasive distribution range of *Diospyros lotus*. Our data do not contradict the hypothesis of a relatively high level of genotype variation of alien species becoming invasive.

Conflict of interest

The authors have no conflicts of interest to declare.

Ethical statements

This article does not contain any studies that would require an ethical statement.

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