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Evaluation of *Lonicera caerulea* L. genotypes based on morphological characteristics of fruits germplasm collection

Olga GRYGORIEVA[1,*], Svitlana KLYMENKO[1], Alla KUKLINA[2], Yulia VINOGRADOVA[2], Olena VERGUN[1], Vladimíra HORČINOVÁ SEDLÁČKOVÁ[3], Ján BRINDZA[3]
[1]Department of Fruit Plants Acclimatization, M.M. Gryshko National Botanical Garden of Ukraine National Academy of Sciences, Kyiv, Ukraine
[2]Main Botanical Garden of Russian Academy of Sciences, Moscow, Russia
[3]Institute of Biodiversity Conservation and Biosafety, Faculty of Agrobiology and Food Resources, Slovak University of Agriculture, Nitra, Slovak Republic

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Abstract: It is known that a polymorphic population consisting of a large number of genotypes adapts more rapidly to the environment conditions. The availability of a large gene pool predetermines the success of the introduction and further breeding of new cultivars with economically valuable features. Morphometrics is a classical tool for studying the amplitude of the variability of cultivated plants and evaluating the genotypic diversity of the artificial population. The objective of this study was to evaluate the morphological parameters of *Lonicera caerulea* L. fruits for 26 genotypes from the population introduced in the M.M. Gryshko National Botanical Garden (Kyiv). Morphological characteristics (weight, width, and length) were analyzed in *L. caerulea* fruits using multivariate analysis. Evaluated morphometric parameters were the following: fruit weight from 0.73 to 1.60 g, fruit length from 16.42 to 27.29 mm, fruit diameter from 7.77 to 12.34 mm. The shape indexes of fruits varied from 1.51 to 3.52. The fruit weight (14.09%–34.50%) and fruit diameter (6.68%–22.76%) are the most valuable characteristics important for further selection. According to their three properties, the 26 genotypes of *L. caerulea* grouped into two groups and five subgroups. Using the PC analysis (PC1 = 71.60%, PC2 = 25.16%, and PC3 = 3.23%), *L. caerulea* genotypes were separated into groups with similar morphological parameters.

Key words: *Lonicera caerulea*, genotypes, fruits, variability

1. Introduction

The genus *Lonicera* L. (Caprifoliaceae Juss.) includes about 200 species, growing in Holarctic temperate. *Lonicera caerulea* L. s.l. is widely cultivated nowadays as an edible plant. This is polymorphic species and is regarded by some authors (Skvortsov, 1986; Kuklina et al., 2012; Kuklina, 2017), as a complex of microspecies or geographical races, including *L. altaica* Pall., *L. pallasii* Ledeb., *L. × subarctica* Pojark., *L. edulis* Turcz. ex Freyn, *L. stenantha* Pojark., *L. buschiorum* Pojark., *L. baltica* Pojark., *L. turczaninowii* Pojark. and *L. kamtschatica* (Sevast.) Pojark.

Significant progress has been made in the industrial cultivation of *Lonicera caerulea* and their processing in China (Huo et al., 2005; Zhao et al., 2015; Wang et al., 2016). Numerous studies are underway in European countries, such as Czech Republic (Antalikova and Matuskovic, 2006), Estonia (Arus and Kask, 2007), Poland (Dziedzic, 2008; Małodobry et al., 2010; Smolik et al., 2010; Ochmian et al., 2012; Kaczmarska et al., 2014), Slovakia (Jurikova et al., 2012a), Romania (Truta et al., 2013), Lithuania (Naugzemys et al., 2014).

L. caerulea fruits are used as fresh or processed in numerous products such as jam, marmalade, jelly, compote, cake, juice, sauce, extracts, liqueurs, smoothie, and wines (Liu et al., 2010; Boyarskikh, 2017; Senica et al., 2019).

L. caerulea is valued for ultraearly fruit ripening, as well as a high content of biologically active phenolic compounds (Khattab et al., 215; Peng et al., 2016; Kucharska et al., 2017) with antioxidant (Bąkowska-Barczak et al., 2007; Gruia et al., 2008; Celli et al., 2014; Gao et al., 2016; Hsu et al., 2016; Lee et al., 2019), antiinflammatory (Xu et al., 2007; Hsu et al., 2016), immunomodulating (Svarcova et al., 2007), antibacterial (Celli et al., 2014; Shi et al., 2016), antiviral (Svarcova et al., 2007), antifungal (Palikova et al., 2008), antiallergic (Svarcova et al., 2007) properties. It is used in medicine, cosmetics, and food industry.

It is believed that *L. caerulea* has been used in folk medicine to reduce the risk of hypertension, glaucoma,

* Correspondence: olgrygorieva@gmail.com

heart attack, anemia, malaria, osteoporosis, gastrointestinal diseases (Anikina et al., 1988; Thompson and Barney, 2007) and diuretic remedies, antiseptic agent and treatment of throat and eyes (Jurikova et al., 2012b).

L. caerulea gene pool available in Kyiv is characterized by a wide range of variability and, therefore, has the significant genotypic potential for further selection for adaptability and improvement of fruit quality.

2. Materials and methods

2.1. Fruit collection

The objects of the research were 10–15-year-old plants of *Lonicera caerulea*, which are growing in the Forest-Steppe of Ukraine in M.M. Gryshko National Botanical Garden of NAS of Ukraine (NBG). The observations of the collection genotypes of *L. caerulea* in the period 2018 were performed during mass fruiting. We have described 26 genotypes (referred LC-01 to LC-26) of *L. caerulea* species.

2.2. Morphometric characteristics

The ripened fruits were harvested in the maturity stage. Pomological characteristics analysis was conducted with four replications on a total of 120 fruits per genotype. In our experiments, only one plant was used per genotype. A total of 3120 fully ripened fruits of *L. caerulea* fruits were investigated. The following measurements were taken: fruit weight, in g, fruit length, in mm, and fruit diameter, in mm. The maximum length and maximum diameter of the fruits were measured using a digital calliper Kronos KM-DSM-200 (0-200/0.01; ±0.02 мм). The fresh fruit weights were determined using analytical scales (Kern ADB-A01S05, Germany).

2.3. Statistical analyses

The biometric data of four quantitative traits were subjected to statistical analyses. Microsoft Excel and STATISTICA 5.5 were used to calculate numerical characteristics such as sample size (n), range (minimum and maximum), arithmetic means value, standard deviation (SD), and coefficient of variation (CV) of a trait. Level of variability was determined as per Stehlíková (1988). Pearson's correlation coefficient was used to depict the relationship between the two traits. Hierarchical cluster analyses of similarity between phenotypes were computed by the Bray-Curtis similarity index and performed using PAST 2.17. Principal component analysis (PCA) was performed to evaluate relationships among variables and some possible genotype groupings based on similar properties by using XLSTAT procedure (XLSTAT 7.5, Addinsoft, USA).

3. Results and discussion

3.1. Fruit pomological properties

Our collection includes more than 40 genotypes of seed origin from European Russia, Kuril, and Canada. The differences in weight, shape, size, the color of fruits, and the degree of the wax coating were noted (Figures 1 and 2). Mature fruits are dark blue, nearly black. The shape of fruits may be ovate, narrowly oblong, broadly oblong, obovate, campanulate; the shape of the calyx is rounded, truncate, acute.

Figure 1. Variability in the shape of *Lonicera caerulea* L. fruits.

Figure 2. Variability of the fruit's distal tip of *Lonicera caerulea* L. Means in columns followed by different letters are different at p = 0.05. Each value represents the mean of three independent experiments (±SD).

Scientific research using morphometric methods is the main way to assess intraspecific variability. Morphometrics, the quantitative approach to the study of morphological variation, combines measurable tools for the description and also statistical analysis of many important aspects of plant organism. Thus, modern approaches in plant breeding based on the evaluation of characteristics among genetic resources and a combination of those in one cultivar (Rohlf and Marcus, 1993; Henderson, 2006).

The biometric values for the weight, length, diameter, and shape index of fruit in the twenty-six *L. caerulea* genotypes are shown in Table 1.

Variation limits for fruit length varied between 8.47 mm for genotype LC-23 and 35.97 mm for genotype LC-09 (Table 1). The value of diameter varied within the interval from 4.92 mm (LC-03) to 15.50 mm (LC-10). Fruit weight, economically, the most important characteristic, ranged from 0.73 (LC-05) to 1.60 g (LC-13).

The average weight of the fruits was determined in the range of 0.73 (LC-05) to 1.60 (LC-13) g (Figure 3), fruit length from 16.42 (LC-24) to 27.29 (LC-26) mm, fruit diameter from 7.77 (LC-26) to 12.34 (LC-16) mm (Figure 4).

These results have shown that fruit weight values are similar within those obtained by Thompson and Barney (2007), Plekhanova (2000), Gawronski et al. (2014) but higher against other authors such as Boyarskikh (2017), Fu et al. (2011), Kulikova (2017), MacKenzie et al. (2018), and Holubec et al. (2019) (Table 2).

According to the literature data, Fu et al. (2011) determined the length of fruits in the range of 11.16 to 19.43 mm, Senica et al. (2018) detected values in the interval of 18.10 to 26.32 mm. Investigations of Holubec et al. (2019) established the range of fruit length of varieties from 15.50 to 20.40 mm. Our data was higher than the results obtained by the last authors.

The shape of each object can be characterized by the shape index, i.e. the length to width ratio. Figure 5 represents the shape index (average values) of fruits, which is ranged from 1.51 (LC-21) to 3.52 (LC-26). Fruit weight and size are primarily phenotypic features and reflect the impact of environmental growth conditions, while the fruit shape index is a genetically fixed feature. It is on this basis that some races and subspecies of *L. caerulea* were previously identified as distinct species (Poyarkova, 1958).

The analysis of the coefficient of variation showed the significant variability of morphological signs between *L. caerulea* samples. The variation coefficients (%) ranged between 14.09 (LC-20) and 34.50 (LC-17) for fruit weight, between 6.91 mm (LC-20) and 17.04 (LC-03) for fruit length, between 6.68 (LC-21) and 22.76 (LC-10) for fruit diameter, and between 6.44 (LC-18) and 16.66 (LC-10) for the shape index (Figure 6). Data showed that the most variable important selection signs are the fruit weight and fruit diameter. These results indicate the promise of breeding in this way of investigation.

3.2. Cluster and principal component analysis

The cluster analysis on the morphological characteristics has been carried out earlier for studying the genetic variability of some other plant species (Henderson, 2006; Kaczmarska et al., 2014; Al-Ruqaie et al., 2016; Krishnapillai and Wijeratnam, 2016) and may also be used as useful tools for accessions screening (Jaćimović, 2015; Ivanišová et al., 2017; Vinogradova et al., 2017; Horčinová Sedláčková et al., 2019).

Based on data and Figure 7, it could be considered that cluster analysis separates *L. caerulea* selections into two main groups, which, in turn, are divided into five subgroups.

The Subgroup I has consisted of the genotype (LC-26), which was found to be most far from all other genotypes Subgroups II, III, and differs from other ones

Table 1. Variation limits of fruits of *Lonicera caerulea* L. genotypes.

Genotypes	Fruit weight (g)		Fruit length (mm)		Fruit diameter (mm)		Shape index	
	min	max	min	max	min	max	min	max
LC-01	0.55	1.66	15.82	28.15	7.42	11.24	1.68	3.15
LC-02	0.49	1.46	12.80	23.95	7.20	11.98	1.44	2.34
LC-03	0.35	1.39	12.92	27.17	4.92	11.11	2.28	3.07
LC-04	0.64	1.51	16.87	26.68	7.39	12.34	1.71	2.87
LC-05	0.38	1.02	13.20	21.05	6.28	10.67	1.38	2.79
LC-06	0.91	1.97	21.22	32.96	9.78	14.18	1.70	3.00
LC-07	0.60	1.62	17.66	26.86	8.39	14.26	1.42	2.55
LC-08	0.69	1,91	18.53	29.74	7.90	13.50	1.66	2.79
LC-09	0.59	1.40	20.58	35.97	7.47	11.82	2.03	3.63
LC-10	0.68	2.05	16.47	31.65	8.36	15.50	1.45	2.99
LC-11	0.60	1.62	21.0	30.16	7,0	13.28	1.98	3.44
LC-12	0.51	1.14	15.73	25.87	6.83	9.93	2.06	2.93
LC-13	0.91	2.32	19.64	30.53	8.54	12.96	1.88	3.07
LC-14	0.24	1.46	15.25	30.52	6.51	11.51	1.61	3.28
LC-15	0.79	1.93	16.52	28.55	9.53	13.87	1.47	2.20
LC-16	0.74	2.33	20.43	32.30	9.35	14.48	1.75	2.42
LC-17	0.43	1.93	16.29	25.24	6.04	10.49	2.00	3.53
LC-18	0.40	1.22	12.28	21.62	7.33	11.21	1.62	2.21
LC-19	0.84	1.82	16.33	21.94	8.61	12.78	1.45	2.17
LC-20	0.70	1.20	14.32	18.72	8.28	11.75	1.29	2.16
LC-21	0.64	1.36	12.59	21.21	8.85	12.57	1.22	2.39
LC-22	0.53	1.29	13.67	22.22	7.93	12.43	1.35	2.08
LC-23	0.59	1.41	8.47	22.34	7.48	11.94	0.86	2.38
LC-24	0.61	1.23	13.18	19.56	8.13	11.21	1.53	2.10
LC-25	0.69	1.18	9.45	20.78	7.43	10.16	0.98	2.28
LC-26	0.53	1.20	22.22	32.30	6.23	10.11	2.76	3.95

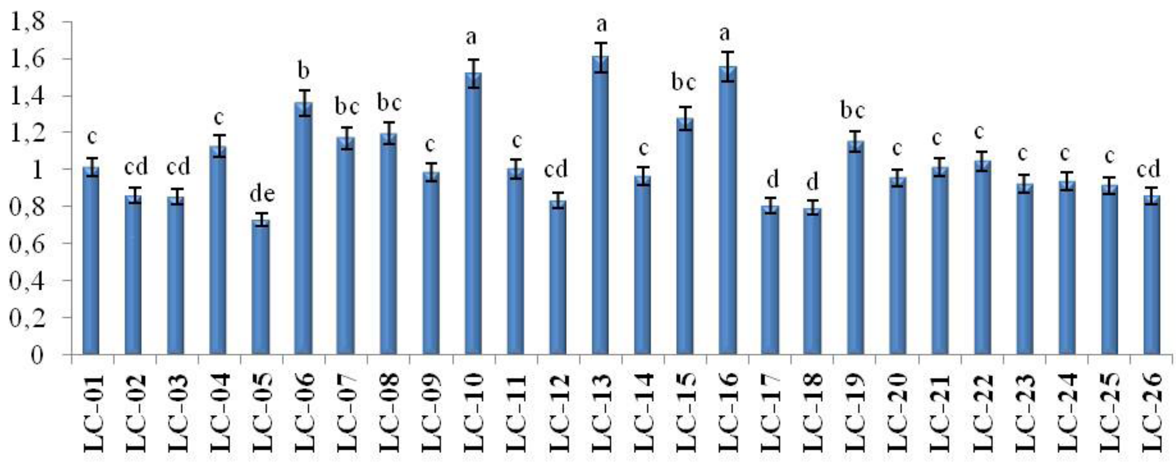

Figure 3. Mean values fruits weight of *Lonicera caerulea* L. genotypes (g). Means in columns followed by different letters are different at p = 0.05. Each value represents the mean of three independent experiments (±SD).

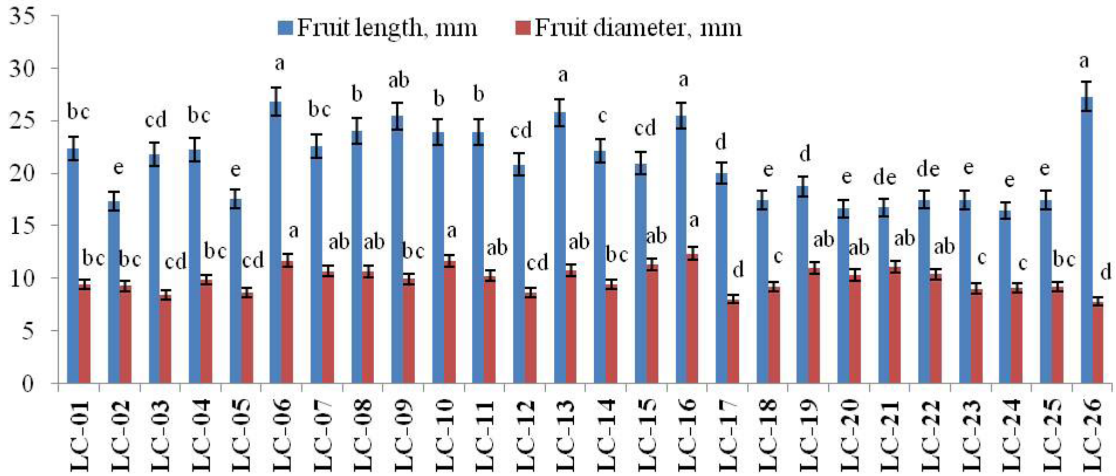

Figure 4. Mean values for various morphometric parameters of fruits of *Lonicera caerulea* L. genotypes. Means in columns followed by different letters are different at p = 0.05. Each value represents the mean of three independent experiments (±SD).

Table 2. Variability of some morphometric characteristics of *Lonicera caerulea* L. fruits, according to the authors from different countries.

Authors	Fruit weight (g)	Fruit length (mm)	Fruit diameter (mm)
Plekhanova (2000)	0.70–1.32	–*	–*
Thompson and Barney (2007)	0.50–2.70	–*	–*
Fu et al. (2011)	0.37–1.01	11.16–19.43	7.05–11.06
Gawronski et al. (2014)	0.56–1.75	–*	–*
Boyarskikh (2017)	0.21–0.80	–*	–*
Kulikova (2017)	0.42–0.63	–*	–*
MacKenzie et al. (2018)	0.38–0.88	–*	–*
Senica et al. (2018)	–*	18.10–26.32	9.90–13.49
Holubec et al. (2019)	0.80–1.50	15.50–20.40	9.0–11.20

Note: –* no data.

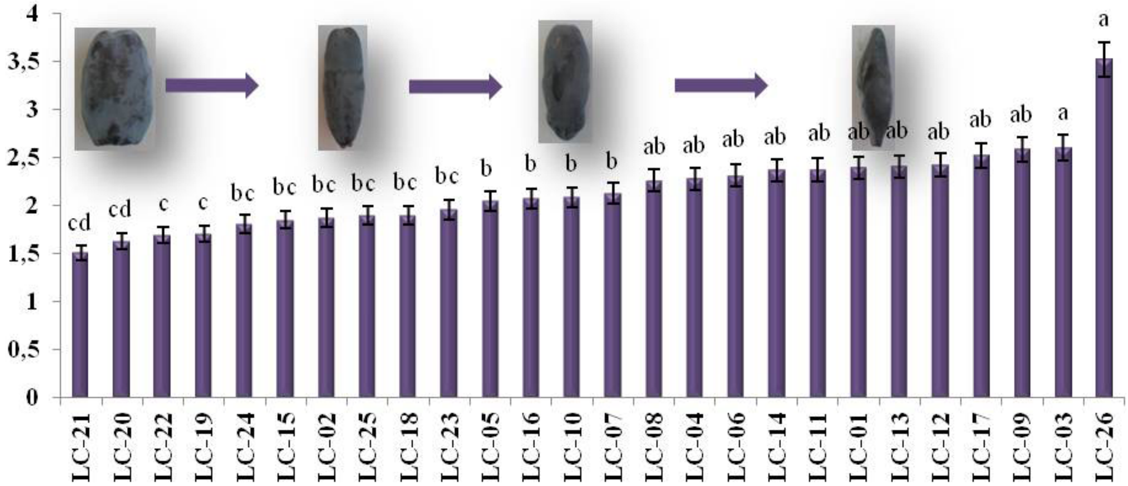

Figure 5. Comparison of the tested *Lonicera caerulea* L. genotypes in the shape index of fruits.

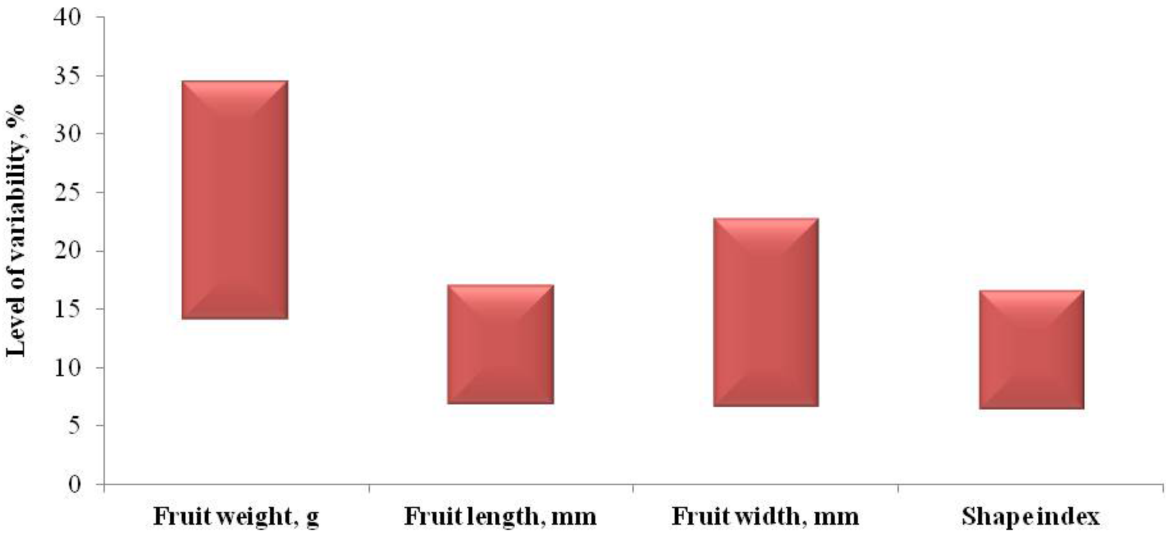

Figure 6. Level of the variability of morphological characters of *Lonicera caerulea* L. fruits (%).

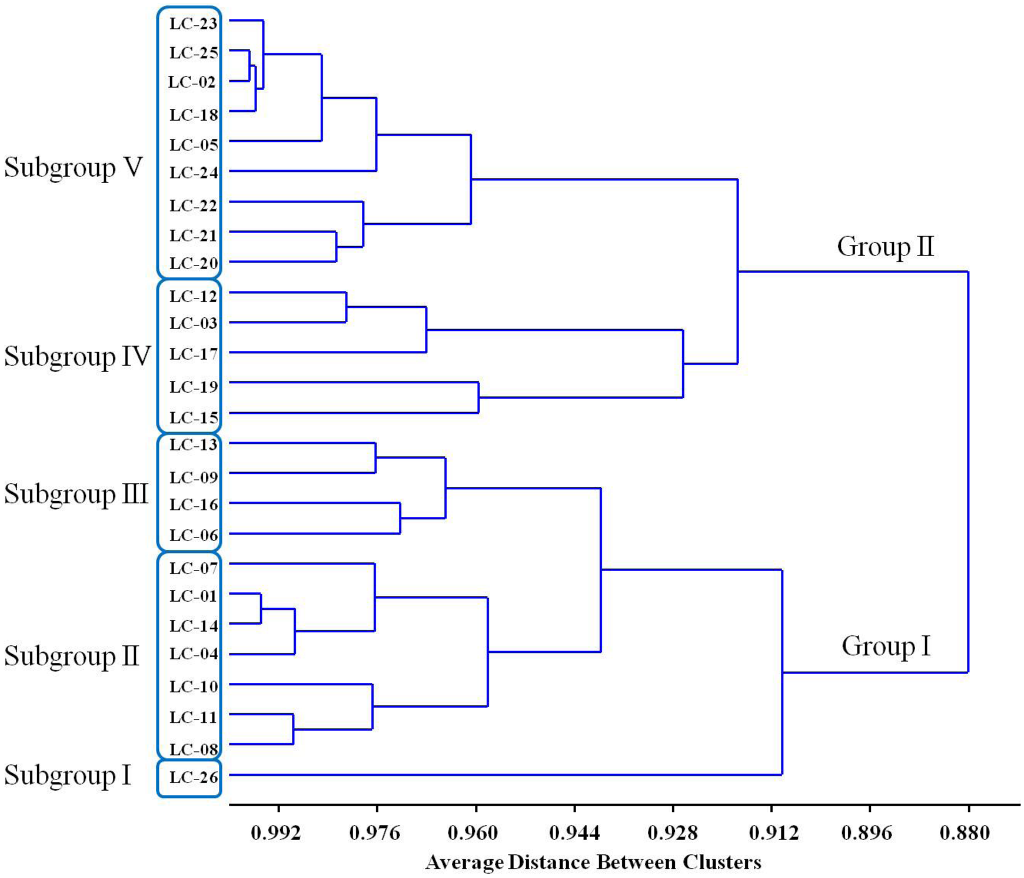

Figure 7. Cluster dendrogram analyzed on three morphometric parameters of fruits of 26 genotypes of *Lonicera caerulea* L.

in all parameters. Subgroup II includes seven genotypes, which had the smallest length of fruits (22.10–24.00 mm) comparing with genotypes of Subgroup III (25.42–26.80 mm).

Group II was further subdivided into two subgroups. The Subgroup IV includes five genotypes which had the larger length fruits (18.73–21.79 mm) as compared with genotypes of Subgroup V (16.42–17.56 mm).

More detailed relationships between perspective genotypes were revealed by PCA. The PCA used in our work showed that 100.00% of the variability observed explained by the first three components (Table 3). PC1, PC2, and PC3 accounted for 71.60%, 25.16%, and 3.23%, respectively. PC1 was positively correlated with all three parameters (Table 3). PC2 was positively correlated with fruit length, whereas fruit weight and fruit width showed a very low negative correlation.

Positive values for PC1 correspond to the genotypes with higher fruit weight, fruit length, and fruit width, as shown in Figure 8. Genotypes LC-06, LC-10, LC-13, and

Table 3. Eigenvalues and proportion of total variability, eigenvectors of the first three principal components (PC), and component scores for 26 genotypes of *Lonicera caerulea* L.

Selection	Component scores		
	PC1	PC2	PC3
LC-01	−0.198	0.549	−0.021
LC-02	−1.386	−0.534	−0.027
LC-03	−1.230	0.928	−0.082
LC-04	0.320	0.279	−0.166
LC-05	−2.024	−0.144	0.087
LC-06	2.507	0.467	0.407
LC-07	0.907	−0.030	0.149
LC-08	1.152	0.314	0.171
LC-09	0.381	1.094	0.565
LC-10	2.551	−0.306	−0.336
LC-11	0.372	0.591	0.531
LC-12	−1.310	0.619	−0.009
LC-13	2.568	0.508	−0.943
LC-14	−0.375	0.513	0.103
LC-15	1.292	−0.768	0.013
LC-16	3.209	−0.254	0.037
LC-17	−1.825	0.703	−0.311
LC-18	−1.603	−0.433	0.147
LC-19	0.501	−1.109	0.081
LC-20	−0.689	−1.210	0.168
LC-21	−0.102	−1.559	0.408
LC-22	−0.271	−1.091	−0.003
LC-23	−1.318	−0.438	−0.338
LC-24	−1.370	−0.729	−0.419
LC-25	−1.241	−0.524	−0.198
LC-26	−0.818	2.563	−0.016
Eigenvalue	2.148	0.755	0.097
Variance (%)	71.602	25.162	3.236
Cumulative	71.602	96.764	100.00
Variable	Component loadings		
	PC1, λ = 71.6	PC2, λ = 25.16	PC3, λ = 3.23
Fruit weight. g	0.967	−0.106	−0.231
Fruit length. mm	0.671	0.736	0.083
Fruit width. mm	0.873	−0.449	0.192

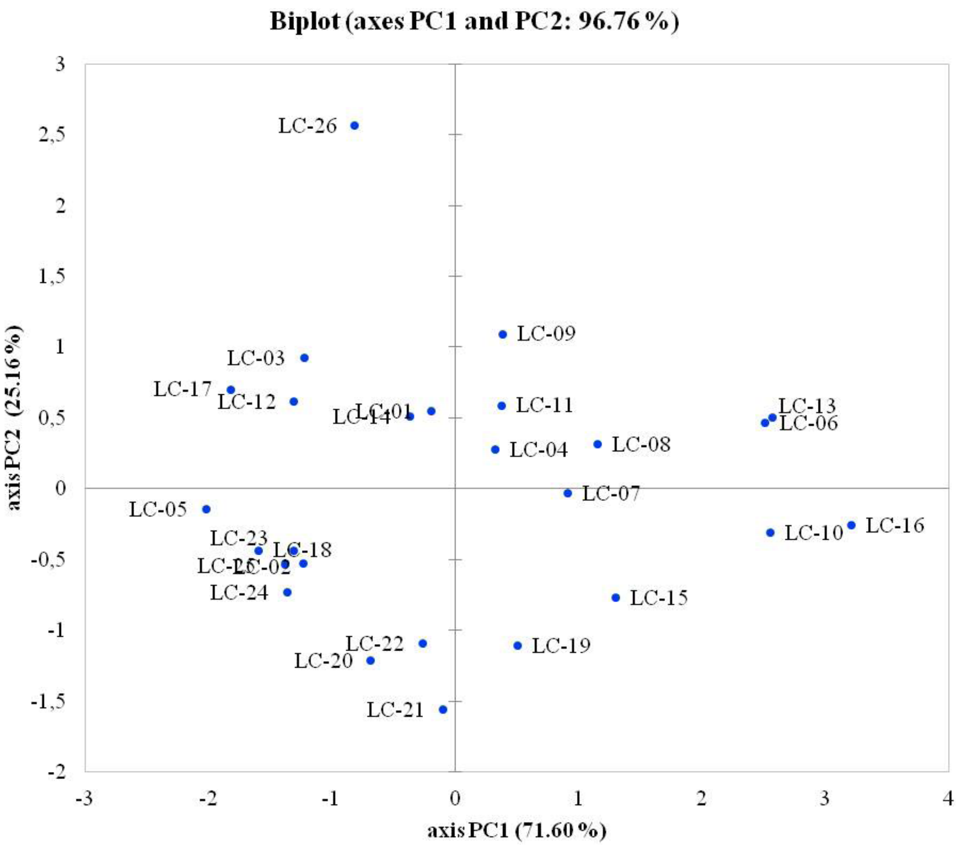

Figure 8. Biplot based on principal components analysis (PCA) for fruit characteristics of 26 genotypes of *Lonicera caerulea* L.

LC-16 were included in this group. The highest negative values for PC1 indicate the genotypes with the smallest fruit weight. This group includes genotypes LC-05, LC-17, and LC-18 (Figure 8). The genotype LC-26 which has the highest PC2 value stands out especially due to the highest fruit weight. The lower negative PC3 value indicates the smallest fruit diameter. These characteristics were observed in genotypes LC-12, LC-22, and LC-26 (Figure 8).

In generally, PC analysis may help to select a set of genotypes with better fruit quality performances (Azodanlou et al., 2003; Mratinić et al., 2011; Milošević et al., 2014; Angmo et al., 2017), which, in our study, might be indicated in LC-06, LC-13, LC-16, and LC-26.

4. Conclusion

The following significant differences among the studied genotypes were noted: weight of fruits from 0.35 to 2.33 g (in 7 times), length of fruits from 8.47 to 35.97 mm (in 4 times), diameter of fruits from 4.92 to 15.50 mm (in 3 times), shape index from 0.86 to 3.95 (in 5 times). The cluster analysis and analysis of the main components demonstrated the importance of the morphometrics for genotype differentiation and/or the further selection of *L. caerulea* in terms of dimensional properties. The size and shape index of the *L. caerulea* fruits can be used to distinguish between genotypes and cultivars, as well as to determine the parameters of postharvest treatment and berry sorting. These data can also be useful for future breeding of new cultivars. The high amplitude of variability in the morphometric characteristics of fruits in the introduction population reflects its high potential stability. The *L. caerulea* collection in the M.M. Gryshko National Botanical Garden of NAS of Ukraine can be assessed as a national gene pool for the conservation of genetic diversity of the valuable fruit crops.

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