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Original paper

Determination of morphological characteristics of leaves in cornelian cherry (*Cornus mas L.*)

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Abstract The characteristics of leaves, such as length, width and foliar surface, can vary between different genotypes within the same species, influencing the growth of plants but also their productivity. This paper presents a model of cornelian cherry leaf research (*Cornus mas* L.) using the ImageJ program. The obtained results indicate high variations for leaf surface characteristics. Thus, the leaf surface had an average value of between 15.32 cm² (C10) and 30.89 cm² (C1), the leaf length between 6.12 cm (C8) and 9.56 cm (C1), the leaf width between 3.57 cm (C10) and 5.64 cm (C1), the length of petiole varied between 2.37 mm (C7) and 4.11 mm (C10). The results obtained can be useful in studies of physiology, plant taxonomy and research on the effects of environmental factors and contribute with more information to complete morphological knowledge concerning this species.

Keywords *Cornus mas L*, leaf surface, leaf dimensions.

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Introduction

Cornelian cherry grows in Romania in deciduous forests on the hills and mountain areas, but we also find it as an ornamental plant, in parks and gardens, due to the early flowering and its special appearance (CORNESCU FRATUTU and COSMULESCU, 2019). The leaves ensure a permanent decoration, in shape, size and appearance and play an important role in the life of trees, as they receive light, thus influencing physiological processes such as photosynthesis, transpiration and absorption (MOUSAVI et al, 2014). The leaves are in opposition, with a short pedicel, oval shaped, 3-5 cm wide and 6-8 cm long, with an entire margin that is shortly acuminate and supplied with visible parallel veins (DA RONCH et al, 2016). Leaf size and shape may vary between different genotypes of the same species. Morphological and morphometrical features such as the ovality ratio, the finesse of tip, the membranous texture, the venation type and notophyll size class allow *Cornus mas* to be adaptable for its temperate even with air humidity and semi-arid regions (BERCU, 2013). To research the biology of fruit trees, precise methods of estimating the surface of leaves are often needed (SPANN and HEEREMA, 2010). Achieved knowledge on traits variability was utilized to develop new and modify already existing descriptors to create an innovated cornelian cherry genotype evaluation descriptor list (BRINDZA et al, 2006). Recently, cornelian cherry has attracted the attention of fruit growers, due to its food value but also ornamental (ERCÝSLÝ, 2004; DA RONCH et al, 2016; COSMULESCU et al, 2019). There are many other specialized papers that provide information on leaf length and width, leaf surface, in several fruit species (HORSLEY, KERSTIENS and HAWES, 1994; CENTRITTO et al, 2000, KERAMATLOU et al, 2015; COSMULESCU et al, 2020). Phenotypic characteristics are also the first to be used for diversity research (KIANI et al, 2010). A high coefficient of variation for morphological characteristics can be an important criterion for selecting superior genotypes. Farmanpour Kalalagh et al (2016) showed that leaf length and width were important features in separating cornelian cherry genotypes in Iran. The purpose of this paper is to highlight the features of *Cornus mas* leaf and to contribute with more information to complete morphological knowledge concerning this species.

Materials and Methods

1. Materials

Observations and measurements were made on a set of 20 mature cornelian cherry leaves (*Cornus mas* L), in 17 cornelian cherry genotypes found in Calaparu population, Gorj County (44°41′2″N 23°19′28″E). Genotypes were coded with letters filled with numeric characters, respectively, C1 ... C17.

2. Methods

Aiming to determine the morphometric characteristics of leaves, the ImageJ program was used to processing and analysing the images (Figure 1), which enabled the measurement of foliar limbus length, width of foliar limbus, petiole length and surface area foliar limbus. Leaves were photographed using a smart phone camera and saved onto a PC computer. Files were then uploaded into ImageJ software Leaf Image Analysis – Surface Area protocol (Figure 1) and the surface area of leaves was determined. Each leaf was measured using an area contour calibrated to 0.01 cm², using the method described by Cosmulescu et al (2020). Based on the obtained dimensions, the length / width ratio of foliar limbus was also calculated, and a classification of genotypes was made by size classes, according to leaf surface area using the model described by Bercu (2013). Measurements made with the ImageJ software enable making a set of statistical measurements of an image. Depending on the selection conducted, the application calculates and displays results of statistical calculation in a table.

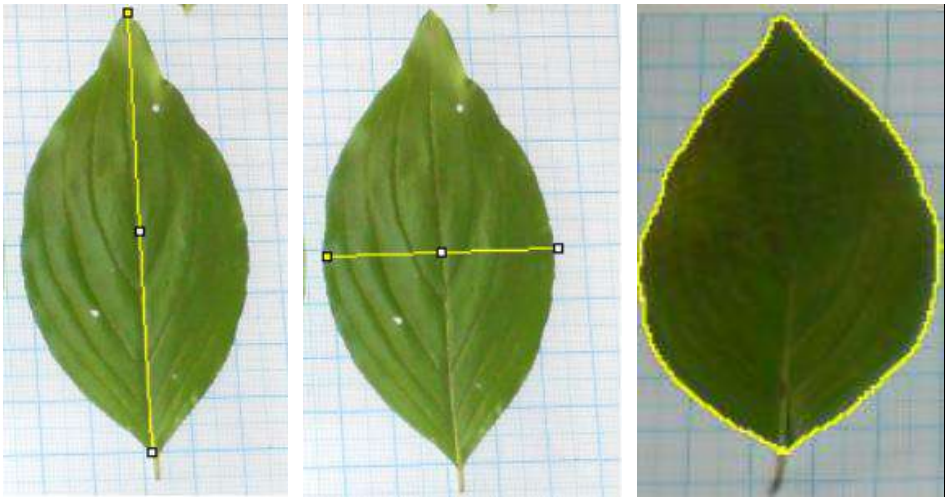

Figure 1. Analysis of cornelian cherry leaves' characteristics

3. Statistical analysis

The data obtained were statistically processed using the program Data analysis in Microsoft Excel (StatPoint Technologies, Warrenton, VA, USA).

Results and Discussions

The knowledge of morphometric characteristics of leaves is of particular importance in the process of selection of new genotypes of cornelian cherry, being known that the leaf is the most important organ of plants, due to its structure that reflects an expression of ability to adapt the plants to ever changing climatic conditions (SANCHEZ, 1967).

Thus in Table 1 are presented the average values of the foliar limbus surface area which varied between 15.32 cm² (C10) and 30.89 cm² (C1), the limits of variation between 11.31 cm² (C3) and 58.62 cm² (C1). The data obtained are comparable to those of the specialized literature. Sotiropoulos et al (2011) reported foliar limbus surface area variation limits from 21.84 cm² to 39.50 cm², while Bercu (2013) found genotypes with leaf surface area between 5.22 cm² and 25.27 cm². The lowest coefficient of variation calculated for leaf surface area, which was 15.21% in C4, and the highest, respectively 36.19%, in C1. Depending on the average value of foliar limbus surface area obtained for each genotype, a classification of them by classes of leaf sizes was made. Thus, of the 17 cornelian cherry genotypes, eight genotypes (C3, C4, C7, C8, C9, C10, C11 and C16) have very small leaves with an average surface area of foliar limbus value between 15.32 cm² (C10) and 19.46 cm² (C16), while the rest of genotypes have small leaves, with an average value of foliar limbus surface area ranging from 20.93 cm² (C6) to 30.89 cm² (C1). The results obtained are similar to those obtained by Bercu (2013), who specified that the studied genotypes were classified as having very small leaves (5.22-19.87 cm²) and having small leaves (20.95-25.27 cm²).

Table 1. Surface area of foliar limbus in genotypes analyzed

Parameters analysed		Genotypes																
		C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15	C16	C17
Surface area of foliar limbus (cm^2)	Average ± SD	30.89± 11.18	26.94 ± 5.52	16.04 ± 3.30	18.58 ± 2.82	20.73 ± 6.04	20.93 ± 6.28	19.22 ± 5.80	15.80 ± 2.54	18.09 ± 4.38	15.32 ± 2.40	16.66 ± 2.65	30.38 ± 8.21	29.92 ± 8.66	27.35 ± 8.36	24.10 ± 3.85	19.46 ± 4.34	27.35 ± 8.21
	Variation range	13.30-58.62	17.0-37.48	11.31-24.37	13.28-23.15	12.83-31.89	12.86-31.89	9.86 - 31.18	11.44-19.20	11.34-25.83	11.57-19.07	13.38-23.13	15.93-41.39	18.48-45.79	14.95-41.05	18.4 - 30.36	13.85-28.67	15.88-41.76
	CV %	36.19	20.51	20.61	15.21	29.14	30	30.2	16.1	24.25	15.71	15.94	27.04	28.96	30.56	15.98	22.3	30.01

Regarding the morphological characteristics, such as the foliar limbus length and width, the ratio beween length and width, and the petiole's length, we can also observe variations between genotypes (Table 2).

Table 2. Length, width and ratio between length and width of leaves from genotypes in Calapăru* population

Parameters analysed		Genotypes																
		C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15	C16	C17
Leaf length (L; cm)	Average ±SD	9.56 ±1.12	8.44 ±0.56	6.61 ±0.54	6.92 ±0.61	7.41 ±1.09	7.41 ±1.08	7.02 ±0.68	6.12 ±0.54	7.64 ±0.90	6.62 ±0.53	7.21 ±0.55	8.85 ±0.93	9.43 ±1.18	9.1 ±1.36	8.19 ±0.88	7.33 ±1.06	8.44 ±1.02
	Variation range	8.14-11.80	7.38 - 9.24	5.93-8.24	5.67 - 7.98	5.84 - 9.15	5.84 - 9.15	5.96 - 8	5.06 - 6.88	5.70 - 8.78	5.54 - 7.7	6.46-8.25	7.3-10.37	7.94-12.14	7.12-11.58	7.18-10.58	5.97-9.37	6.98-10.75
	CV%	11.8	6.64	8.3	8.95	14.73	14.66	9.75	8.8	12.15	8.03	7.66	10.5	12.54	15	10.8	14.55	12.09
Leaf width (W; cm)	Average ±SD	5.64± 1.01	4.82 ±0.63	3.62± 0.52	4.13 ±0.38	4.23 ±0.67	4.22 ±0.66	4.13 ±0.84	4.04 ±0.51	4.08 ±0.60	3.57 ±0.41	3.66 ± 0.34	5.12 ± 0.88	4.99 ± 0.85	4.77 ± 0.76	4.82± 0.46	4.06± 0.38	4.87 ±0.81
	Variation range	4.04 - 7.50	3.49-5.92	2.97-4.62	3.42-4.86	3.37-5.44	3.37-5.44	2.62-5.48	3.16-4.82	2.98-4.94	2.9-4.36	3.06 - 4.53	3.43 - 6.78	3.94 - 6.61	3.32 - 5.91	4.18 - 5.59	3.43-4.67	3.59-6.37
	CV%	17.88	13.05	14.4	9.4	15.8	15.8	20.31	12.65	14.71	11.69	9.4	17.2	17.05	16.09	9.65	9.38	16.61
L/W (cm)	Average ±SD	1.72± 0.20	1.77 ±0.17	1.85± 0.24	1.68 ±0.15	1.76 ±0.14	1.76 ±0.14	1.76 ±0.35	1.55 ±0.19	1.84 ±0.14	1.87 ±0.23	1.98 ± 0.21	1.75 ± 0.20	1.90 ± 0.14	1.92 ± 0.17	1.71 ± 0.17	1.81 ± 0.21	1.75 ±0.16
	Variation range	1.35 - 2.05	1.47-2.19	1.42-2.24	1.40-2.05	1.51-2.07	1.52-2.07	1.4-2.41	1.32-1.90	1.60-2.20	1.44-2.33	1.72 - 2.39	1.51 - 2.26	1.58 - 2.15	1.54 - 2.30	1.39 - 2.00	1.37 - 2.09	1.51-2.15
	CV%	11.42	9.8	12.71	9.15	7.81	7.84	19.69	12.26	7.68	12.24	10.5	11.48	7.22	8.98	9.79	11.46	8.97
Petiole length (mm)	Average ±SD	3.53± 1.16	3.52 ±1.05	3.56± 1.04	3.52 ±0.97	3.74 ±0.91	2.74 ±0.94	2.37 ±0.59	3.2 ±1.06	3.05 ±0.86	4.11 ±0.77	2.96 ± 0.57	3.33 ± 0.64	3.29 ± 0.82	3.37 ± 0.65	3.23 ± 0.50	3.78 ± 1.09	3.23 ±0.61
	Variation range	2.00 - 6.00	2.00 - 5.3	2.00-6.00	2.00 - 5.2	2.10-5.00	1.9 - 4.7	1.90-4.00	1.9-6.00	2.00-5.00	2.9-5.3	2.2 - 4.1	2.3 - 4.5	2.00 - 5.00	2.2 - 4.1	2.00 - 4.1	1.9 - 6.2	2.2 - 4.3
	CV%	32.88	29.87	29.31	27.73	24.55	34.38	24.94	33.27	28.47	18.76	19.58	19.24	24.94	19.42	15.72	28.8	18.98

The average value of foliar limbus length ranged from 6.12 cm (C8) to 9.56 cm (C1). The lowest value of foliar limbus length 5.06 cm was measured in C8 genotype, while the highest (11.80 cm) was in C1 genotype. The lowest coefficient of variation for length was obtained in C3 genotype (6.64%) while the highest was in C14 genotype (15%). The results obtained are higher than those obtained by Bercu (2013), respectively 39-80 mm and Bosancic (2009), respectively, 43.84-54.87 mm. Farmanpour Kalalagh et al (2016) found for the foliar limbus length, values

between 77.09 and 105.7 mm. The width of leaves studied had an average value between 3.57 cm (C11) and 5.64 cm (C1), with variation limits ranging from 2.62 cm (C7) to 7.50 cm (C1), and with a variation coefficient between 9.38% (C16) and 20.37% (C7). Compared to the literature, the data obtained for the width of foliar limbus are higher than those obtained by Bosancic (2009), respectively, 21.05-26.07 mm, and by Bercu (2013), respectively, 20-49 mm, but lower than the average value found by Sotiropoulos et al (2011), respectively, 58.42 mm. For petiole length, the average value varied between 2.37 mm (C7) and 4.11 mm (C10), with variation limits between 1.90 mm (C6, C7, C8) and 6.20 mm (C16) and with a variation coefficient with values between 15.72% (C15) and 34.38% (C6). Previous studies have shown an average petiole length of 5-7 mm (SOTIROPOULOS et al, 2011), 7.88 mm (MOROZOWSKA et al, 2013) and 6.07 mm (FARMANPOUR KALALAGH et al, 2016). The leaf length/width ratio plays an important role in determining leaf shape and had an average value comprised between 1.55 (C8) and 1.98 (C11). The variation limits for leaf shape ratio ranged from 1.32 (C8) to 2.41 (C7). The results obtained from this report indicate an oval or elongated leaf shape. The coefficients of determination (R^2) and correlation (r) between leaf dimensions and surface area of foliar limbus were also calculated. Thus, the coefficient of determination between length and surface area of foliar limbus had values between 0.242 (C10) and 0.932 (C14), and the one between width and surface area of foliar limbus was between 0.495 (C15) and 0.971 (C17) (Table 3).

Table 3. Correlation coefficient between length, width and surface area of limbus

Parameters analysed	Linear equation	Determination coefficient (R^2) and correlation coefficient (r)	Genotypes																
			C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15	C16	C17
Between length and surface area of limbus	y=5.347x-19.39	R^2	0.67	0.63	0.41	0.57	0.84	0.87	0.41	0.60	0.78	0.24	0.36	0.83	0.92	0.93	0.43	0.75	0.82
	R^2=0.795	r	0.82	0.80	0.65	0.76	0.92	0.93	0.64	0.78	0.89	0.49	0.60	0.91	0.96	0.97	0.66	0.87	0.91
Between width and surface area of limbus	y=8.666x-15.22	R^2	0.83	0.86	0.91	0.54	0.96	0.95	0.91	0.52	0.76	0.79	0.70	0.90	0.90	0.85	0.49	0.75	0.97
	R^2=0.868	r	0.91	0.93	0.96	0.74	0.97	0.98	0.96	0.73	0.87	0.89	0.84	0.95	0.95	0.92	0.70	0.87	0.99

There is a positive correlation, so the two correlated variables vary in the same direction. Regarding the correlation coefficient between length and surface area of foliar limbus, values between r=0.49 in C10 genotype and r = 0.97 in C5 genotype were obtained. The correlation coefficient between width and surface area of foliar limbus varied between 0.70-0.99 for C15 and C17 genotypes, respectively. The values obtained from calculations, support the fact that there is a close correlation between leaves characteristics; these are in accordance with those in the specialized literature obtained for different fruit species: $r^2 = 0.99$ for walnut (KERAMATLOU et al, 2015) and $r^2 = 0.93$ for medlar (COSMULESCU et al, 2020).

Conclusions

As a result of the obtained results, we can observe that the foliar limbus has recorded higher values of its length, smaller values of its width, and the length / width ratio indicates an oval-elliptic leaf shape, with a relatively short petiole. The leaves of the analyzed genotypes were included in the small class and very small class. The results obtained can be useful for future research on the influence of specific environmental conditions on leaf characteristics, in the plant taxonomy and the evolution of the species *Cornus mas L.*

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