https://doi.org/10.17221/97/2019-HORTSCI

Determination of leaf characteristics in different medlar genotypes using the ImageJ program

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Citation: Cosmulescu S., Scrieciu F., Manda M. (2020): Determination of leaf characteristics in different medlar genotypes using the ImageJ. Hort. Sci. (Prague), 47: 117–121.

Abstract: The size and shape of leaves can vary significantly between different genotypes within the same species and they implicitly influence plant growth and therefore productivity. The aim of this study was to compare the length, width, and surface area of leaf in nine medlar genotypes (*Mespilus germanica* L.) through image segmentation procedure using ImageJ software. The data indicate large variations for leaf surface area characteristics from one genotype to another, 2.12 fold for leaf surface area (22.95–48.8 cm^2), 1.38 times for leaf length (8.8–12.18 cm) and 1.6 times for leaf width (3.5–5.60 cm). Leaf shape and leaf surface area vary between different genotypes analysed, and the method used can represent a good, non-destructive model of quick and reliable estimation of the medlar leaves surface area. The information obtained can be used in physiology studies, regardless of genetic material.

Keywords: *Mespilus germanica* L.; genotypes; leaf area; leaf dimensions

Foliage surface of trees determines the interception of light, and it is an indicator of productivity and is frequently used in many horticultural research experiments. It varies by species, cultivar, age and planting distance. Previous research has shown that the foliage surface is influencing the main physiological processes, such as photosynthesis, transpiration and absorption (Flore 1994). In addition, it is known that leaf size and shape can vary significantly between different genotypes within the same species (Cristofori et al. 2007) and they implicitly influence plant growth and therefore productivity (Pérez-Pastor et al. 2014). Also, morphological characteristics of leaves can be an important parameter in appreciating the decorative potential of spontaneous and cultivated plants, given the appearance of the plant that can be influenced by the shape and size of the leaf. Recently, the importance of using double-breeding, food and ornamental species in the green spaces has increased. Medlar (*Mespilus germanica* L.) is also included in this group of popular fruit trees. Medlar has been cultivated for many years in countries in Europe and Asia for both edible fruits and ornamental qualities (Cosmulescu et al. 2018), but quite rarely, mainly in botanical gardens or small farms (Grygorieva et al. 2018). Several studies have been conducted on the parameters of medlar leaf. Morphological features of medlar have ornamental value, the leaf being one of them. Mendoza-De Gyves et al. (2008) have set-up a model for quick estimation of leaf surface area in medlar, based on leaf parameters. Various other papers provide information on length and width of leaf, the foliar surface, in several fruit tree species: avocado (Uzun,

https://doi.org/10.17221/97/2019-HORTSCI

Celik 1999), pistachios (Ranjbar, Damme 1999), cherry tree (Demirsoy, Demirsoy et al. 2004), chestnut (Serdar, Demirsoy 2006), apple (Kishore et al. 2012) and hazelnut (Cristofori et al. 2007). The aim of this study was to compare the length, width and surface area of in nine medlar (*Mespilus germanica* L.) genotypes through the image segmentation procedure using ImageJ software, the information obtained having relevance in physiology studies.

MATERIAL AND METHODS

Material. Medlar leaves (*Mespilus germanica* L.) were taken under the study in nine genotypes from different areas of Romania and considered to be relevant in terms of morphological characteristics of tree and fruit. The biotypes were encoded taking their names from the place where they have been identified, such as: M1, M2, M3 (Măstăsari; 44°51'N, 23°5'E), Cr1, Cr2 (Croici; 44°49'53''N, 23°5'50''E), N1 (Nanov; 43°59'11''N 25°18'5''E), C1 (Craiova; 44°20'N, 23°49'E), T1 (Turnu-Ruieni; 45°23'07''N, 22°23'05''E), E1 (Ezeriș; 45°23'58''N, 21°52'37''E).

Method. The ImageJ software, an image processing and analysis program (Figure 1), was used to determine the leaf surface area. For all selected genotypes, 20 leaves were taken from each genotype, from the annual shoots (the sixth leaf). These were used to calculate the leaf surface area. The leaves of varied sizes (from large to small) were selected at a distance from the ground between 1 m and 2 m, during the summer season (July 2018). Leaves were photographed using a smart phone camera and saved into a 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 cm^2. Research on fruit tree biology often requires accurate and precise estimation of the foliar surface area (Spann, Heerema 2010). Measurements made with the ImageJ software enable to make 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.

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

RESULTS AND DISCUSSION

The surface area of medlar leaves is an important characteristic in the selection of new genotypes in terms of productivity, taking into account that one of the main indices of photosynthetic activity in fruit tree plants is the foliar surface area, but also for decorative characteristics. Table 1 shows the variability of average values in leaf surface area, the standard error, the minimum and maximum values, and also the variation coefficient of the studied genotypes. The data indicate large variations for leaf characteristics from one genotype to another, a large difference (2.12 times) for the leaf surface area in genotypes identified in localities Nanov and Turnu-Ruieni (22.95–48.8 cm^2), of 2.8 times higher for the standard error (M2: 2.07 and T1 respectively: 0.72) and 1.39 times higher for the variation coefficient (M2: 19.79, T1: 14.19).

Figure 1. Leaf visual aspect of medlar genotypes used to develop leaf characteristics

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Table 1. Measured mean, minimum and maximum values of leaf area for leaves of medlar genotypes*

Parameters analysed		Characteristics genotype								
		C1	N1	M1	M2	M3	T1	E1	Cr1	Cr2
Leaf area (cm^2)	mean ± SE	29.76 ± 1.22	48.8 ± 1.84	32.17± 1.35	36.77 ± 2.07	35.56 ± 1.21	22.95 ± 0.72	29.31 ± 1.11	25.40 ± 0.85	27.45 ± 1.01
	min. / max.	18.6 / 38.52	35.28 / 64.08	23.7 / 44	24.40 / 58.84	27.95 / 47.12	17.90 / 28.43	19.63 / 36.42	21.68 / 34.31	18.08 / 35.2
	CV (%)	18.37	16.90	18.58	19.79	15.28	14.19	17.07	15.12	16.54

*n = 20 leaves for each genotype; data are mean values ± standard error; CV – coefficient of variance

Table 2. Measured mean, minimum and maximum values of length, width and length: width ratio for leaves of medlar genotypes*

Parameters analysed		Genotype								
		C1	N1	M1	M2	M3	T1	E1	Cr1	Cr2
Leaf length (cm)	mean ± SE	9.7 ± 0.3	12.18 ± 0.26	11.24 ± 0.20	10.7 ± 0.32	11.02 ± 0.21	8.8 ± 0.16	9.92 ± 0.24	9.20 ± 0.15	9.56 ± 0.21
	min./max.	6.77 / 12.3	9.27 / 13.76	9.82 / 12.86	7.75 / 13.98	9.59 / 12.72	7.75 / 10.57	7.95 / 11.31	8.17 / 0.66	7.56 / 10.72
	CV%	13.89	9.58	8.13	13.41	8.72	8.53	10.97	7.44	10.12
Leaf width (cm)	mean ± SE	3.96 ± 0.10	5.60 ± 0.16	3.94 ± 0.11	4.9 ± 0.15	4.42 ±0.10	3.5 ± 0.08	4.13 ± 0.08	3.89 ± 0.11	3.97 ± 0.07
	min. / max.	2.95 / 4.65	4.32 / 6.81	3.3 / 5.01	3.99 / 5.69	3.52 / 5.66	2.85 / 4.11	3.42 / 4.8	3.18 / 4.84	3.2 / 4.59
	CV%	11.89	13.18	12.85	14.02	10.28	11.33	9.61	12.71	8.85
Length / width ratio	mean ± SE	2.44 ± 0.10	2.17 ± 0.08	2.85 ± 0.07	2.18 ± 0.12	2.49 ± 0.07	2.51± 0.08	2.40 ± 0.04	2.36 ± 0.06	2.40 ± 0.04
	min. / max.	1.96/3.71	1.73 / 2.84	2.37 / 3.41	0.67 / 3.36	1.94 / 3.19	2.12 / 3.36	2.13 / 2.75	1.99 / 2.78	2.10 / 2.75
	CV (%)	17.62	16.12	10.52	14.67	13.25	13.94	7.08	10.59	7.50

*n = 20 leaves for each genotype, data are mean values ± standard error; CV – coefficient of variance

Table 3. Coefficient of determination (R^2) and coefficient of corelation (r) for the leaf area and the independent variables length, width, for leaves of medlar ($P < 0.0001$)

Parameters analysed	Linear equation	R^2 and r value	Genotype								
			C1	N1	M1	M2	M3	T1	E1	Cr1	Cr2
Between length and leaf area	$y = 5.0593x - 19.902$ $R^2 = 0.6305$	R^2	0.65	0.25	0.33	0.30	0.37	0.37	0.82	0.38	0.73
		r	0.80	0.5	0.57	0.54	0.6	0.6	0.9	0.61	0.85
Between width and leaf area	$y = 0.0769x + 1.7982$ $R^2 = 0.8385$	R^2	0.51	0.75	0.80	0.76	0.61	0.66	0.87	0.79	0.86
		r	0.71	0.86	0.89	0.87	0.78	0.81	0.93	0.88	0.92

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Depending on this characteristic, medlar genotypes can be grouped as follows: large leaves (over 40 cm², N1 genotype), medium leaves (30–40 cm², M1, M2, M3 genotype) and small (less than 30 cm², C1, T1, E1, Cr1, Cr2 genotype). Variability of leaf surface area is characteristic for fruit tree species. A variation in the leaf surface area was also found in the apple tree, from 33.47 cm², during the fruit-setting period, up to 44.31 cm² during the growth period (Balan 2010). Leaf length and width are also characteristics that differentiate between genotypes. Regarding the mean leaf length, the highest value was recorded at in N1 genotype (12.18 cm), while the lowest was found in T1 genotype (8.8 cm). For leaf width, the average value varied within fairly large limits. Thus, the highest value was obtained in N1 genotype (5.60 cm), followed by genotypes M2 (4.9 cm), M3 (4.42 cm), E1 (4.13 cm), while the lowest value was 3.5 cm in T1 genotype. The leaf shape expressed as the ratio of leaf length and width (L : W) ranged from 2.17 to 2.85 (Table 2). Depending on the L : W ratio, medlar genotypes were divided into three groups. The first group included N1, M2, and Cr1 genotypes, which were characterised and well-documented by wide leaves (L : W ratio of 2.17, 2.18 and 2.36, respectively). The second group comprises C1, E1 and Cr2, T1, M3 genotypes, outlined by leaves of intermediate shape (L : W ratio ranging between 2.49 and 2.51), while the third group was formed of single genotype (M1) that recorded a narrow leaf shape (L : W ratio of 2.85). Similar studies by Mendoza-de Gyves et al. (2008) on the ratio between length and width of leaves in *Mespilus germanica* L. showed that the ratio varied from 2.12 to 2.60, the max. value being less than in (M1) genotype identified in Mătăsari area (2.85). To illustrate the correlation between leaf dimensions (length, width) and leaf surface area, the determination coefficient (R^2) and correlation coefficient (r) were calculated. Determination coefficient between leaf length and surface area varied between 0.253 and 0.825, while the coefficient between leaf width and surface area was between 0.518 and 0.873 (Table 3). There is a direct positive correlation: the two correlated variables are varying in the same way. Thus, with respect to the correlation coefficient between leaf length and surface area area, a reasonable correlation was found (r = 0.5, 0.54, 0.57) in N1, M2 and T1 genotypes, a high correlation (r = 0.6; 0.61) was found in T1, M3 and Cr1 genotypes, and, respectively, a very high correlation (r = 0.8; 0.85; 0.9), ie a very close relationship between variables in C1, Cr2 genotypes, respectively E1. In terms of the correlation coefficient between the leaf width and surface area, it varied between 0.71–0.92, thus resulting in a high correlation, respectively, a very high correlation. Correlation coefficients calculated show that there is a close correlation between laminar area, length and width. The results obtained are consistent with those obtained in other fruit tree species as well: $r2$ = 0.955 for the cherry tree (Demirsoy et al. 2003), $r2$ = 0.997 for citrus (Mazzini et al. 2010), $r2$ = 0.99 for walnut (Keramatlou et al. 2015). In conclusion, leaf characteristics vary between different genotypes analysed, a factor that was established by other authors as well (Stoppani et al. 2003). The method used may be a good, non-destructive model of quick and reliable estimation of medlar leaves area, while the information obtained can be used in physiology studies, regardless of genetic material.

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Received: July 19, 2019
Accepted: September 27, 2019