

Comparative statistical analysis in determining leaf area based on leaf parameters – Case study on poplar

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Abstract

The study comparatively analyzed the leaf area of poplar leaves, determined by analyzing scanned images and based on leaf parameters. Leaf length (L), leaf width (W) and leaf perimeter (Per) were foliar parameters used to determine leaf area by regression analysis. A collection of leaf samples, 150 leaves, was used in the study. Correlation analysis identified a positive correlation, of very high intensity, between foliar parameters and leaf area ($r = 0.946$ to $r = 0.978$), in conditions of statistical safety ($p < 0.001$). By quadratic regression analysis, leaf area was obtained based on foliar parameters, considered two by two in different combinations, with $p < 0.001$. The level of fit between scanned leaf area (LA) and leaf area based on leaf parameters was described by linear equations, with $R^2 = 0.943$ to $R^2 = 0.989$, $p < 0.001$. Comparative analysis of the data series for measured leaf area and that determined based on leaf parameters showed decisive evidence for equality of means (Anova Test), and median values (Kruskal-Wallis test). Dunn's post hoc test confirmed non-significant differences in the comparative analysis of leaf area values determined based on leaf parameters and by analyzing scanned images.

Keywords: Comparative analysis; Dunn's post hoc; Kruskal-Wallis; leaf area; leaf parameters; poplar

Introduction

Leaves are plant organs, with varied shapes and sizes, which depend primarily on the plant species, but also on environmental conditions, on the "plant x environment" interaction as a result of the process of evolution, growth and development of plants [9], [28], [25], [21].

Morphological parameters, structural elements and nutritional levels at the level of plant leaves are important to analyze and understand strategies and mechanisms of adaptation and response that plants have presented to environmental conditions [26], [8]. Variable levels of structure and functionality in plant leaves are reflected in the process of photosynthesis, primary plant productivity, ecosystem functions [23], [16]. The size and functionality of leaves vary, as a response to plant adaptation to environmental factors, in relation to their own resource requirements and metabolic processes [7], [33], [36].

Dimensional elements of leaves and interdependence relationships between foliar parameters, leaf traits and physiological processes have been studied in different plant species [14], [19], [34], [2].

Structural elements of leaves and various aspects of systemic functionality of plants have been considered in urban landscape planning [1]. Foliar parameters of leaves have been studied and analyzed as indicators of adaptation to climate change of some plant species [3].

Leaf area was analyzed in relation to leaf parameters, length (L) and width (W), and characterized based on specific leaf shape elements [12]. Leaf area indices have been studied in relation to biometric plant parameters [2]. Leaf shape was analyzed in relation to leaf length and surface area in different plant species [37], [32]. Variation in leaf shape in relation to tree size was recorded, but no influence was found in the proportional relationship between leaf area and dimensional elements of leaves [22].

The determination of leaf area by non-destructive methods has been promoted through numerous studies on different plant species or agricultural or horticultural crops [18], [20], [39], [6]. Determining leaf area in plants based on the dimensional parameters of leaves (length, width) has found high interest, due to its accessibility, low costs, and sufficiently high accuracy [24], [31], [30]. Various mathematical models have been reported for determining leaf area based on dimensional parameters of leaves [31], [11], [13].

The study aimed to compare the leaf area of poplar leaves obtained by analyzing scanned images, and the leaf area predicted based on the dimensional parameters of the leaves (length - L, width - W, perimeter

- Per) and to assess the level of precision in determining leaf area through the considered procedures.

Material and Method

The biological material was represented by 150 poplar leaves, randomly sampled, of different sizes. Measurements were made with a ruler to record the dimensions of the leaves, length (L, cm) and width (W, cm). The working accuracy was ± 0.5 mm. The leaves were scanned (scanner), in a 1:1 ratio to ensure proportionality of the leaf dimensions.

The scanned images (Fig. 1) were analyzed [27] and values for leaf perimeter (Per) and leaf area (LA, cm^2) were obtained.



Figure 1. Poplar leaf, scanned image, binarized

Descriptive statistical analysis characterized the data series through specific parameters. The distribution of the data series was evaluated. Correspondence analysis was applied to quantify the Eigenvalue level, and to find out the score of the parameters on the correspondence axes.

The interdependence relationship between leaf parameters (L, W, Per) and leaf area (LA) obtained by scanning was evaluated (Pearson's r correlation)

Leaf area (LA) was analyzed in relation to leaf parameters (L, W, and Per) considered two variables in different combinations, within the regression analysis. Data sets for predicted leaf area in relation to L and W, $LA(L,W)$, in relation to L and Per $LA(L,Per)$ and in relation to W and Per $LA(W,Per)$ were obtained. 3D graphical models and isoquant format represented the distribution of LA values in relation to the variables considered in the analysis. Statistical safety parameters used to express the safety of the models and results were the regression coefficient (R^2), the F test, and the statistical safety parameter p .

The level of fit between the predicted leaf area value series, $LA(L,W)$, $LA(L,Per)$, $LA(W,Per)$, and the scanned LA value series was analyzed by linear regression analysis.

A comparative analysis of the predicted LA values, $LA(L,W)$, $LA(L,Per)$, $LA(W,Per)$, with the LA values obtained by scanning was performed, based on specific tests (ANOVA, Kruskal-Wallis, Dunn's post hoc). In relation to the purpose of the study, PAST v4.17 software [10], JASP v0.16.4 software [15], and Mathematica software [35] were used.

Results and Discussion

The data resulting from the analysis of leaf samples, regarding leaf parameters, were statistically analyzed, with the results in Table 1. Leaf parameters varied, for leaf length, $L = 6.20 - 18.20 \pm 0.21$ cm, leaf width $W = 5.50 - 19.65 \pm 0.23$ cm, perimeter $Per = 20.63 - 78.83 \pm 0.99$ cm, respectively leaf area $LA = 19.93 - 259.91 \pm 3.89$ cm^2 . The data series presented normal distribution, with $r = 0.995$ (for L), $r = 0.996$ (for W), $r = 0.992$ (for Per), respectively $r = 0.973$ (for LA), Fig. 2.

Table 1. Results of Descriptive statistical analysis

Statistical parameters	Leaf parameters			
	L	W	Per	LA
N	150	150	150	150
Min	6.20	5.50	20.63	19.93
Max	18.20	19.65	78.33	259.91
Sum	1713.95	1783.70	6848.49	14567.17

Mean	11.43	11.89	45.66	97.11
Std. error	0.21	0.23	0.99	3.89
Variance	6.86	8.11	147.87	2271.22
Stand. dev	2.62	2.85	12.16	47.66
Median	11.48	11.75	45.35	87.22
25 prntil	9.48	9.78	37.00	60.91
75 prntil	13.10	13.83	54.15	125.71
Mode	12.60	12.80	NA	NA
Skewness	0.20	0.25	0.41	0.84
Kurtosis	-0.49	-0.45	-0.03	0.37
Geom. mean	11.12	11.55	44.04	85.94
Coeff. var	22.92	23.95	26.63	49.07

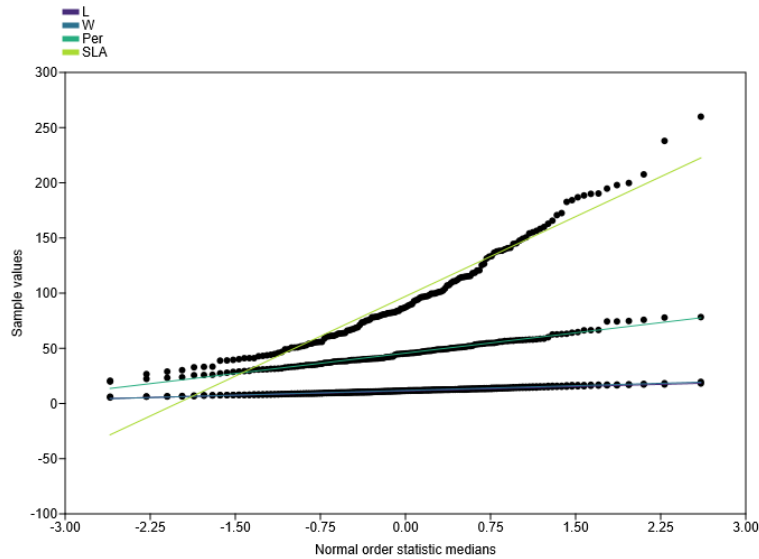


Figure 2. Distribution of data series for leaf parameters

Correspondence analysis explained the Eigenvalue values (Table 2) and the score of the foliar parameters on the axes (Table 3), with the representation of the series of values of the foliar parameters in Fig. 3.

Table 2. Eigenvalue according to Correspondence analysis based on foliar parameters in poplar leaves

Axis	Eigenvalue	% of total	Cumulative
1	0.014365	96.947	96.947
2	0.000277	1.870	98.817
3	0.000175	1.183	100

Table 3. Foliar parameter score on axes, based on Correspondence analysis

Parameters	Axis 1	Axis 2	Axis 3
L	0.16960	0.01737	0.04278
W	0.14458	0.04817	-0.02346
Per	0.13423	-0.01810	-0.00594
SLA	-0.10077	0.00057	0.00063

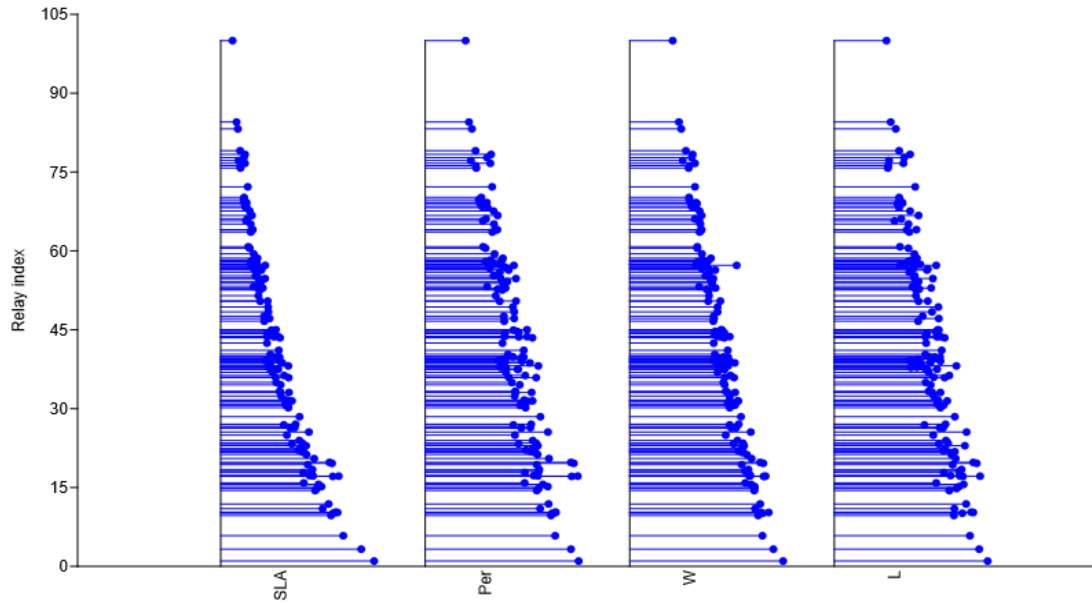


Figure 3. Graphical representation of data series for foliar parameters in poplar leaves

Correlation analysis (Pearson's r) showed the level of interdependence between foliar parameters in poplar leaves. Based on the data series, a positive correlation of very strong intensity was recorded at the level of the studied foliar parameters, in conditions of statistical safety ($p < 0.001$), Table 4.

The distribution of the data series, the graphical expression of the interdependence relationship between foliar parameters, and the confidence interval values are presented in Fig. 4. In the case of the L and W parameters, the correlation level was $r = 0.952$, with a 95% confidence interval (0.934, 0.965). In the case of the L and Per parameters, the correlation level was $r = 0.972$, with a 95% confidence interval (0.962, 0.980). For the parameters L and LA, the correlation level was $r = 0.946$, with a 95% confidence interval (0.927, 0.961). For the parameters W and Per, the correlation level was 0.965, with a 95% confidence interval (0.952, 0.975). For the parameters W and LA, the correlation level was 0.978, with a 95% confidence interval (0.969, 0.984). For the parameters Per and LA, the correlation level was $r = 0.960$, with a 95% confidence interval (0.945, 0.971).

Table 4. Correlation matrix table

Variable	Statistical parameters	L	W	Per	LA
L	Pearson's r	—			
	p-value	—			
W	Pearson's r	0.952***	—		
	p-value	< .001	—		
Per	Pearson's r	0.972***	0.965***	—	
	p-value	< .001	< .001	—	
LA	Pearson's r	0.946***	0.978***	0.960***	—
	p-value	< .001	< .001	< .001	—

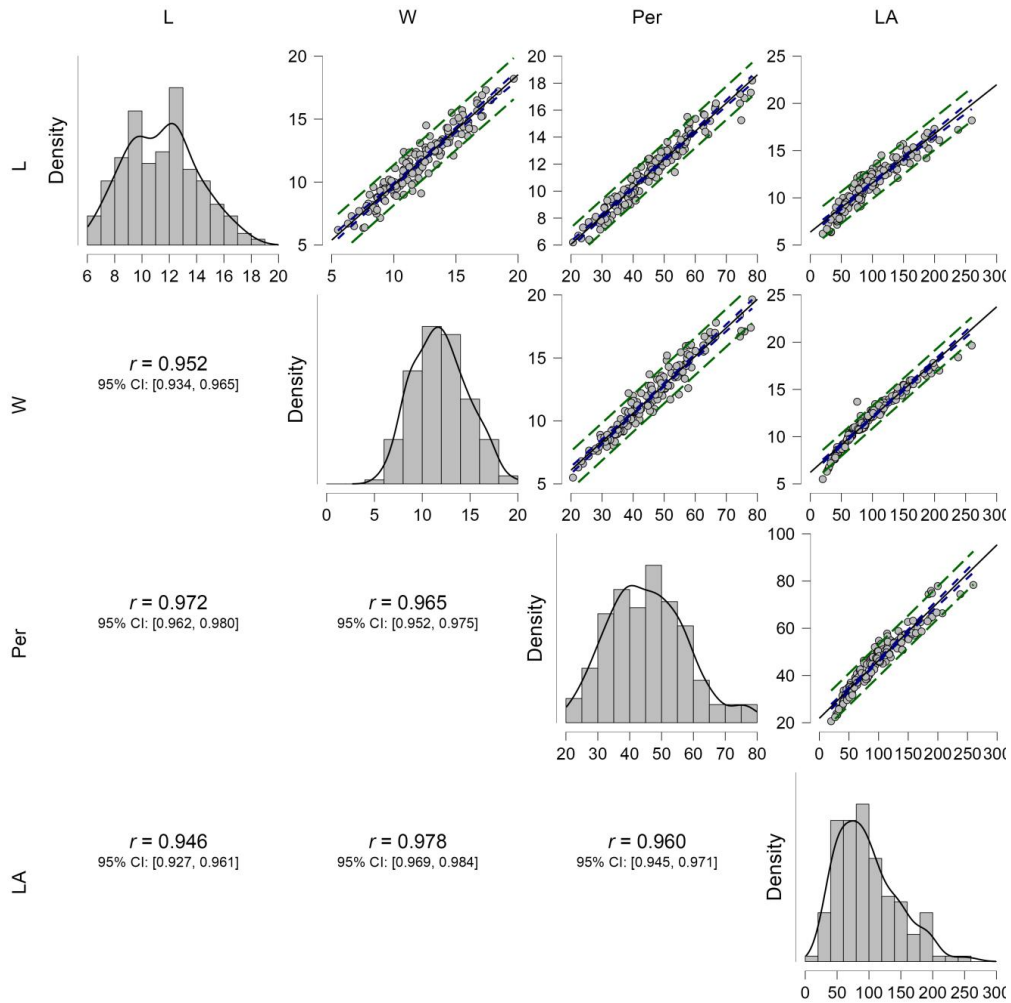


Figure 4. Distribution of data series, interdependence between parameters and confidence interval

Starting from the correlation levels recorded between foliar parameters, regression analysis was applied to find out the level of confidence in estimating leaf area (LA) based on foliar parameters (L, W, Per).

The regression analysis facilitated the estimation of leaf area (LA) based on the foliar parameters L, W and Per, in different combinations. The result was equation (1) as a general model, with the values of the equation coefficients and statistical safety parameters presented in table 5, in relation to the foliar parameters considered in the analysis.

$$LA_{(L,W,Per)} = ax^2 + by^2 + cx + dy + exy + f \quad (1)$$

where: $LA_{(L,W,Per)}$ – predicted leaf area based on leaf parameters; x, y – variables in Eq. (1) according to Table 5; a, b, c, d, e, f – coefficients of Eq. (1), with values according to Table 5

Table 5. The values of the coefficients and statistical parameters related to Equation (1)

Predicted Leaf Area	Equation (1) variables	Values of the equation (1) coefficients						Statistical safety parameters		
		a	b	c	d	e	f	R ²	F	p
$LA_{(L,W)}$	$x = L,$ $y = W$	-0.556478	0.163342	1.984598	-4.111839	1.166661	8.618649	0.984	1727.7 3	<0.001
$LA_{(L,Per)}$	$x = L,$ $y = Per$	-1.926818	-0.151262	-9.790556	1.985103	1.277969	14.569805	0.943	473.17	<0.001
$LA_{(W,Per)}$	$x = W,$ $y = Per$	-1.644400	-0.139105	-1.029509	-0.103633	1.151854	6.649599	0.985	1933.5 1	<0.001

In the case of estimating LA based on the parameters L and W, the regression analysis showed a statistical safety level according to $R^2 = 0.984$, $F = 1727.73$, $p < 0.001$ (Table 5). From the analysis of the coefficient values (Table 5) as well as from the graphic distribution (Fig. 5) the divergent contribution of the two parameters to the formation of the leaf surface was observed.

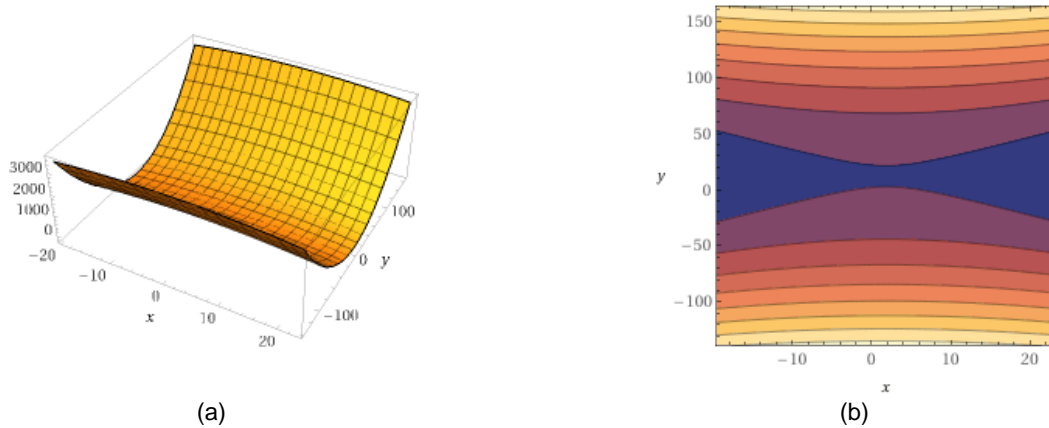


Figure 5. Graphical distribution of LA in relation to L (x – axis) and W (y – axis) in poplar leaves; (a) – 3D model, (b) model in isoquant format

In the case of estimating LA based on the parameters L and Per, the regression analysis showed a statistical safety level according to $R^2 = 0.943$, $F = 473.17$, $p < 0.001$ (Table 5). From the analysis of the coefficient values (Table 5) as well as from the graphic distribution (Fig. 6) the convergent contribution of the two parameters to the formation of the leaf surface was observed.

In the case of estimating LA based on the W and Per parameters, the regression analysis showed a statistical safety level according to $R^2 = 0.985$, $F = 1933.51$, $p < 0.001$ (Table 5). From the analysis of the coefficient values (Table 5) as well as from the graphic distribution (Fig. 7) the convergent contribution of the two parameters to the formation of the leaf area was observed.

The level of fit of predicted leaf area (LA_{pred}) values with actual leaf area values was evaluated by regression analysis. In the case of LA estimated based on L and W, the regression analysis led to linear Equation (2), with $R^2 = 0.989$, $p < 0.001$, with regression line in Fig. 8(a). In the case of LA estimated based on L and Per, the regression analysis led to linear Equation (3), with $R^2 = 0.943$, $p < 0.001$, with regression line in Fig. 8(b). In the case of LA estimated based on W and Per, the regression analysis led to linear Equation (4), with $R^2 = 0.989$, $p < 0.001$, with regression line in Fig. 8(c).

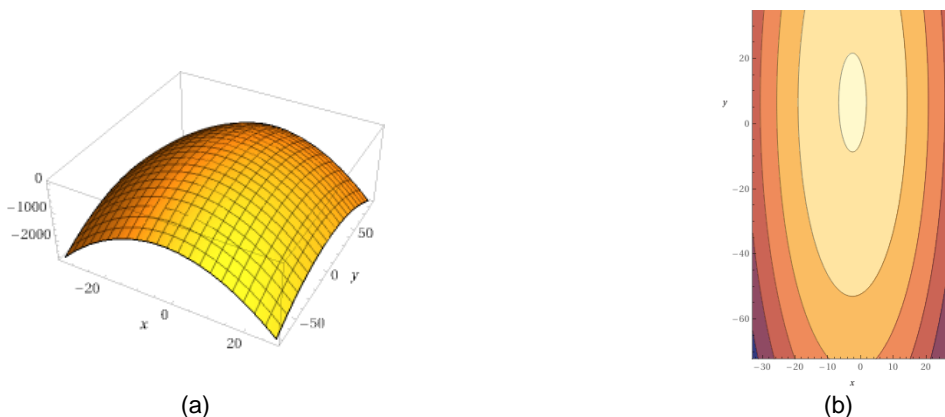


Figure 6. Graphical distribution of LA in relation to L (x – axis) and Per (y – axis) in poplar leaves; (a) – 3D model, (b) model in isoquant format

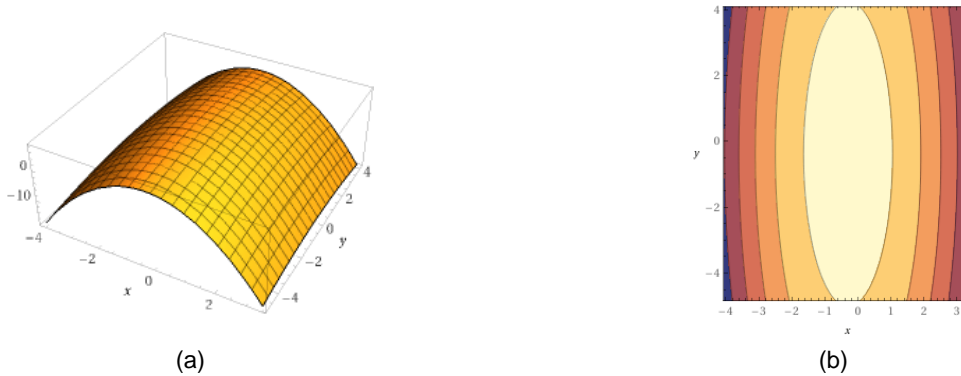


Figure 7. Graphical distribution of LA in relation to W (x – axis) and Per (y – axis) in poplar leaves; (a) – 3D model, (b) model in isoquant format

$$LA_{pred} = 0.9864 x + 1.036 \tag{2}$$

$$LA_{pred} = 0.9427 x + 5.527 \tag{3}$$

$$LA_{pred} = 0.9875 x + 0.993 \tag{4}$$

where: LA_{pred} – predicted Leaf Area (cm^2) based on: L and W, Equation (2); L and Per, Equation (3); W and Per, Equation (4); x – LA real, obtained by scann method (cm^2)

To further evaluate the level of precision and statistical reliability in predicting leaf area based on leaf parameters, the data series for leaf area measured by scanning (LA), and leaf area predicted based on leaf parameters, $LA_{(L,W)}$, $LA_{(L,Per)}$, $LA_{(W,Per)}$ were analyzed. The ANOVA test showed obvious decisiveness for the equality of means, Table 6.

The nonparametric Kruskal-Wallis test showed that there were no significant differences between the median values for the leaf area data series depending on the method of determination (measurement, or predictive based on leaf parameters), according to $H(\chi^2) = 0.03624$, H_c (tie corrected) = 0.03624, $p = 0.9982$ ($p > 0.05$). Dunn's post hoc test confirmed the lack of significance in the comparative analysis of the data series, and the similarity of the leaf area data series, by the methods determined, Table 7.

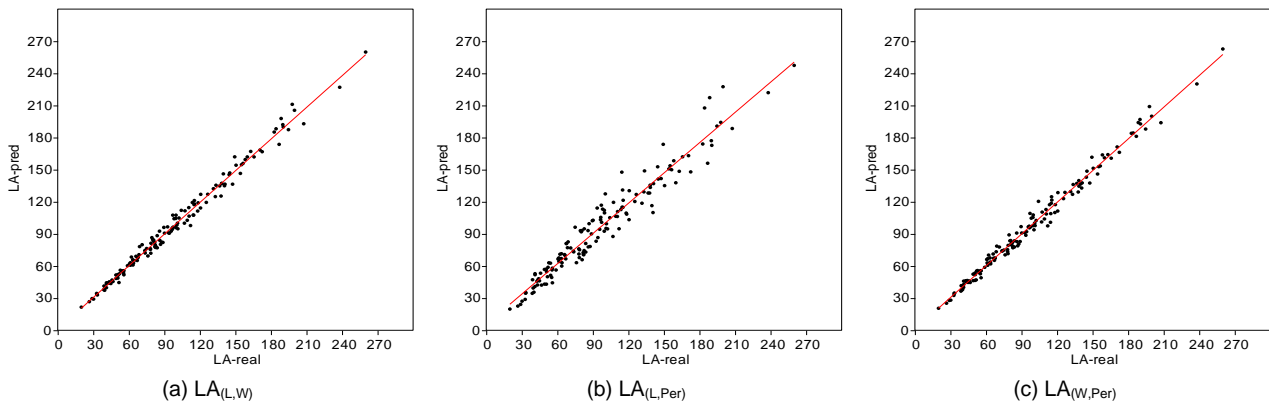


Figure 8. Graphical representation of the fitting line in the comparative analysis of predicted LA values in relation to the real ones

Table 6. ANOVA Test results

Statistical parameters	Sum of sqrs	df	Mean square	F	p (same)
Between groups:	8.89754	3	2.96585	0.00133	0.9999
Within groups:	1.33E+06	596	2223.92		
Total:	1.33E+06	599	1		
Components of variance (only for random effects):					
Var(group):	-14.80640	Var(error):	2223.92	ICC:	-0.00670
omega2:	0				
Welch F test in the case of unequal variances:	F=0.001324, df=331.1, p=0.9999				
Bayes factor:	0.0008292 (decisive evidence for equal means)				

Table 7. Dunn's post hoc test results

	LA	LA _(L,W)	LA _(L,Per)	LA _(W,Per)
LA		0.9503	0.9054	0.9835
LA _(L,W)	0.9503		0.8562	0.9668
LA _(L,Per)	0.9054	0.8562		0.8890
LA _(W,Per)	0.9835	0.9668	0.8890	

More and more studies have promoted the determination of leaf area, the analysis and characterization of plant genotypes based on foliar parameters, as a result of the advantages presented [4], [29], [30]. Some studies used only leaf parameters (length, width) and found certain prediction models for leaf area, and in the case of other studies, additional correction factors were used in order to increase the accuracy of calculating leaf area based on leaf parameters [31], [11], [30]. Leaf area determined based on leaf parameters (L, W) presented differentiated proportional relationships in relation to leaf length and width, but also influenced by leaf shape [31], [38], [30], [17]. In the present study, a higher interdependence relationship was found between leaf area (LA) and leaf width (W), with $r = 0.978^{***}$, than between LA and leaf length (L), with $r = 0.946^{***}$, or between LA and leaf perimeter (Per), with $r = 0.960$. In studies on energy poplar leaves, values for correction coefficients in determining leaf area based on foliar parameters (L, and w) were reported, under conditions of statistical safety [5]. In the regression analysis for leaf area prediction based on leaf parameters, the model that used leaf width (W) and leaf perimeter (Per) generated more accurate values for leaf area, compared to the models that used the other leaf parameters as variables (Table 5). Comparative analysis (Dunn's post hoc test) showed no statistically significant differences between the LA values obtained by scanning or the LA values predicted based on the leaf parameters LA(L,W), LA(L,Per), LA(W,Per), Table 7. Thus, the very close level between the mean values of the leaf area data series obtained was confirmed. The recorded results showed that either method (scanning, or based on the leaf parameters in the tested combinations) led to similar results for the leaf area of poplar leaves.

Conclusions

The data series regarding foliar parameters in poplar leaves presented a normal distribution, according to the values $r = 0.995$ (for L), $r = 0.996$ (for W), $r = 0.992$ (for Per), and $r = 0.973$ (for LA). A very high level of interdependence was recorded between foliar parameters, according to Pearson's r ($p < 0.001$).

Leaf area estimated by quadratic regression analysis based on the parameters L (leaf length), W (leaf width) and Per (leaf perimeter), led to results with statistical certainty ($p < 0.001$). The highest level of precision was recorded when the leaf parameters W and Per were used, with $R^2 = 0.985$, $F = 1933.51$, compared to $R^2 = 0.984$ when using the parameters L and W, respectively $R^2 = 0.943$ when using the parameters L and Per. The level of fit of predicted leaf area based on the leaf parameters and measured leaf area obtained by scanning presented a very high level of statistical certainty in all cases ($p < 0.001$).

Comparative analysis between leaf area data series obtained by scanning (LA) and obtained by estimation based on leaf parameters, LA_(L,W), LA_(L,Per), LA_(W,Per) showed very close levels of mean and median values, with differences without statistical certainty. The results of comparative tests showed that any method of determining leaf area in poplar leaves, based on leaf parameters, can be used with statistical certainty.

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