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VARIABILITY OF SOME PARAMETERS OF THE LEAVES IN LINDEN SHOOTS

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ABSTRACT
The study analyzed the variability of some leaf parameters in linden leaves, Tilia tomentosa Moench. The leaf samples were taken from young shoots, with annual growth, from the area of the Cenad Forest Protected Area, Timis County, Romania. Sample shoots for the study were taken in July. A number of 15 leaves per shoot were analyzed, from the base to the top of the shoot. Petiole length (Pl), petiole diameter (Pd), leaf length (Ll), leaf width (Lw), leaf perimeter (Per) were determined. The following values of the foliar parameters Pl = 1.40-7.90 ± 0.48 cm, Pd = 1.6-4.57 ± 0.24 mm, Ll = 3.90-20.90 ± 1.36 cm, Lw = 4.10-20.40 ± 1.26 cm, respectively Per = 12.58-67.54 ± 4.46 units, were registered. High values of the coefficient of variation (CV) were recorded in the case of the parameter Pl (CV_{Pl} = 40.22945), and low values were recorded in the case of petiole diameter Pd (CV_{Pd} = 25.39963); intermediate values were recorded in the case of the other parameters (CV_{Ll} = 32.91767; CV_{Lw} = 31.05987; CV_{Per} = 31.73974). The Pl / Pd ratio showed superunitary values in the basal leaves and subunitary in the leaves with a middle and terminal position on the shoot. The ratio Ll/Lw presented subunit values for the basal leaves and terminal position on the shoot and superunitary values for those with a middle position. The ratio Per/Ll and Per/Lw showed superunitary values in all leaf samples, regardless of the position on the shoot. Within PCA, PC1 explained 81.459% of variance, and PC2 explained 16.404% of variance. Cluster analysis facilitated the grouping of leaf samples based on similarity, in statistical safety conditions (Coph.corr. = 0.872). The regression analysis facilitated the obtaining of some models, of equation type and graphs (3D and isoquant) regarding the variation Per in relation to Ll and Lw, in conditions of statistical safety (R^2 = 0.999, p <0.001, F = 3617.452).
KEY WORDS: *leaf parameter, models, PCA, Tilia tomentosa Moench., variability*

INTRODUCTION

The features of the leaves of the plants vary in relation to the plant species, the geographical location, the seasons, the position of the leaves on the plants skeletal structure, growth conditions etc. (Datcu *et al*., 2017; Bloomfield *et al*., 2018; Liu *et al*., 2019a; Homeier *et al*., 2021).

Multiple descriptors and investigative techniques have been used for the study, analysis, identification and classification of leaf-based plants (da Silva *et al*., 2015; Thyagharajan and Kiruba Raji, 2019; Chaki *et al*., 2020).

The leaves of the plants, based on specific elements or specific descriptive parameters, have been used to recognize and characterize and classify plant species both by classical, botanical methods and by imaging analysis, such as leaf pattern recognition (Leister *et al*., 1999; Dornbusch *et al*., 2012; Sala *et al*., 2017; Zhang *et al*., 2020).

Imaging analysis has also been used to determine leaf area and of some foliar indices in plants (Bignami and Rossini, 1996; Campillo *et al*., 2010; Marcon *et al*., 2011; Easlon and Bloom, 2014; Sudheer *et al*., 2015; Drienovsky *et al*., 2017a) and determining the degree of pathogenic attack (Arnal Barbedo, 2013; Schikora and Schikora, 2014; Drienovsky *et al*., 2017b; Pavicic *et al*., 2021).

Foliar parameters such as leaf length and width (L, w) have been used in various studies to analyze and characterize leaves in different species and genotypes of plants (Cândea-Crăciun *et al*., 2018; Nakanwagi *et al*., 2018; Shi *et al*., 2019a,b, 2021; Zhang, 2020).

Leaf perimeter has also been used as a specific element of leaf geometry in studies in different plant species (Maloof *et al*., 2013; Zhou *et al*., 2017; Liu *et al*., 2019b). Compared to classical methods, the determination of the perimeter by imaging analysis has multiple advantages, especially that it is fast and accurate (Liu *et al*., 2019b).

To characterize the leaves in relation to different physiological factors or processes, different ratios between geometric elements (length, width, perimeter, leaf area, venation etc.) have also been determined in some studies (Osone *et al*., 2008; Sun *et al*., 2018; Sicard, 2019; Shi *et al*., 2019b, 2021; He *et al*., 2020).

Different mathematical models in the form of equations of variable complexity, or graphical models have been used to describe the variation of foliar geometric parameters in relation to species, genotype, environmental factors, and interdependence relationships between parameters (Wang *et al*., 2013; Coussement *et al*., 2018; Ubbens *et al*., 2018; Wen *et al*., 2018; Yonekura *et al*., 2019).

The present study analyzed several foliar parameters and their variation patterns in the description of linden leaves, *Tilia tomentosa* Moench.

MATERIAL AND METHODS

The study analyzed the variability of some foliar parameters in linden leaves, and evaluated models to describe their variation. Leaf samples were taken from annual growth shoots, from the species *Tilia tomentosa* Moench., from the area of the Cenad Forest Protected Area, Timis County, Romania, figure 1.

Determinations were made on the leaves regarding the petiole length (Pl), petiole diameter (Pd), leaves length (Ll), leaves width (Lw) and leaves perimeter (Per). The values of the parameters Pl, Ll and Lw were obtained by ruler measurement, with an accuracy of ±0.5mm. The values of the Pd parameter were obtained by measurement with electronic caliper, with an accuracy of ±0.001mm. The values of the Per parameter were obtained by analyzing the scanned images of the leaves, 1:1 ratio (Rasband, 1997).

The statistical analysis of the data was done through the ANOVA test, in order to evaluate the presence of the variant and the statistical safety of the set of experimental data.

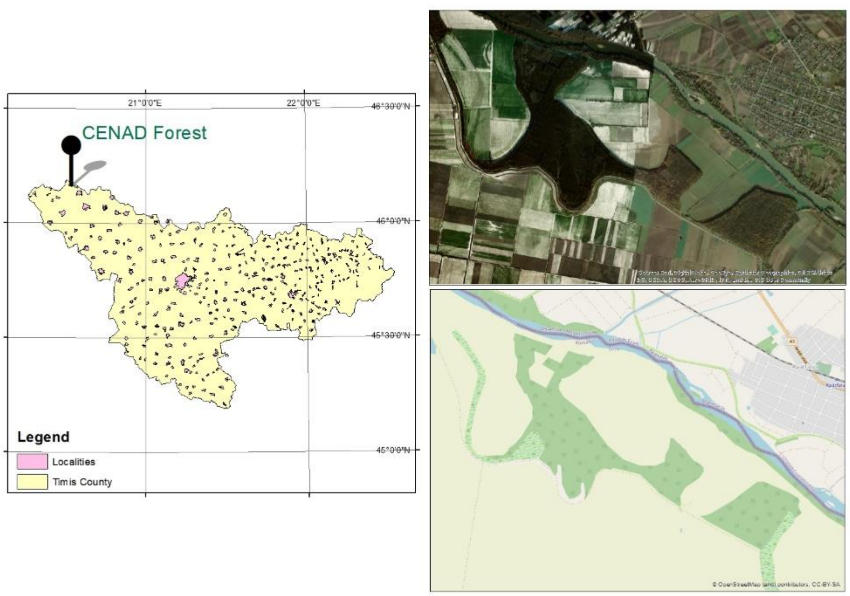

FIGURE 1. Leaf sampling area; Cenad Forest Protected Area, Timis County, Romania

Descriptive Statistical Analysis was performed to evaluate the variability of the foliar parameters studied, based on the coefficient of variation (CV). Graphic analysis of the type Diversity profiles was also used for this purpose. In order to evaluate interdependently between the studied parameters, the correlation analysis was performed. PCA and Cluster Analysis were used to capture the positioning and grouping of the studied cases (leaf sample) in relation to the evaluated parameters, and the degree of similarity. Regression analysis was used to obtain a model to describe the variation of leaf perimeter, in relation to leaves elements (Ll, Lw).

In order to assess the safety of the results in the statistical analysis of the experimental data, well-known statistical parameters were used (Coph. Corr, R^2, r, p). Past software (Hammer *et al*., 2001) and Wolfram Alpha (2020) were used to analyze and interpret data, and generate graphs.

RESULTS AND DISCUSSION

From the analysis of foliar parameters in the samples of linden leaves (*Tilia tomentosa* Moench.) were recorded values for petiole length (Pl) between 1.40 - 7.90 ± 0.48 cm, petiole diameter (Pd) between 1.6-4.57 ±0.24 mm, leaf length (Ll) between 3.90-20.90 ±1.36 cm, leaf width (Lw) between 4.10-20.40 ±1.26 cm, respectively for perimeter (Per) values between 12.58-67.54 ±4.46 units. The graphical representation of the studied foliar parameters, in the form of a box-plot, with the standard error, is represented in figure 2.

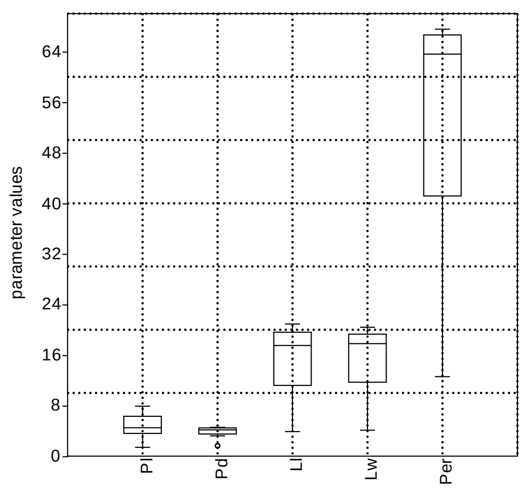

FIGURE 2. Leaf parameters studied in *Tilia tomentosa* Moench. leaves samples, in the form a box-plot representation

Single factor ANOVA test confirmed the presence of variance in the experimental data set and the statistical safety, in $p<0.001$, $F>Fcrit$, for Alpha = 0.001 conditions, table 1.

The statistical analysis showed very strong correlations between the length and width of the leaf, Ll and Lw (r = 0.991). Also, very strong correlations were recorded between Ll and Per (r = 0.987) and between Lw and Per (r = 0.993). Strong correlations were recorded between Pd and Per (r = 0.840), between Pd and Lw (r = 0.856) and between Pd and Ll (r = 0.872). Weak correlations were recorded between Pl and L (r = 0.510), between Pl and Lw (r = 0.579) and between Pl and Per (r = 0.596).

TABLE 1. ANOVA single factor test for the analyzed data

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	25781.35	4	6445.338	90.53494	6.51E-27	5.200847
Within Groups	4983.42	70	71.19172			
Total	30764.77	74				

Alpha=0.001

The degree of heterogeneity of the studied foliar parameters was analyzed in terms of the coefficient of variation (CV). High values of the coefficient of variation were recorded in the case of the parameter Pl (CV_{Pl} = 40.22945), and low values were recorded in the case of petioles diameter Pd (CV_{Pd} = 25.39963). In the case of leaf length (Ll), leaf width (Lw) and leaf perimeter (Per), intermediate values were recorded (CV_{Ll} = 32.91767; CV_{Lw} = 31.05987; CV_{Per} = 31.73974). Graphic analysis of the Diversity profiles type similarly expresses the variation of the values within the studied foliar parameters, figure 3.

Different ratios were calculated between the studied leaf parameters, and the obtained data are presented in table 1. In the case of the Pl/Pd ratio, superunitary values were recorded for the basal leaves (eg 4.0476 in the case of L1; 2.1467 in the case of L2) and subunit values for the leaves at the top of the shoot (eg 0.6231 in the case of L14; 0.8750 in the case of L15). In the case of the ratio Ll/Lw, subunit values were registered in the case of the basal leaves (eg 0.9573 in the case of L1; 0.9689 in the case of L2), as well as in those at the top of the shoot (eg 0.9467 in the case of L14; 0.9512 in the case of L15). In the case of the Per/Ll ratio, superunitary values were registered for all leaves (eg 3.6752 in the case of L1; 3.6690 in the case of L2; 3.5392 in the case of L14; 0.2262 in the case of L15). In the case of the Per/Lw ratio, superunitary values were recorded over the entire length of the shoot (eg 3.5181 in the case of L1; 3.5550 in the case of L2; 3.3504 in the case of L14; 3.0688 in the case of L15).

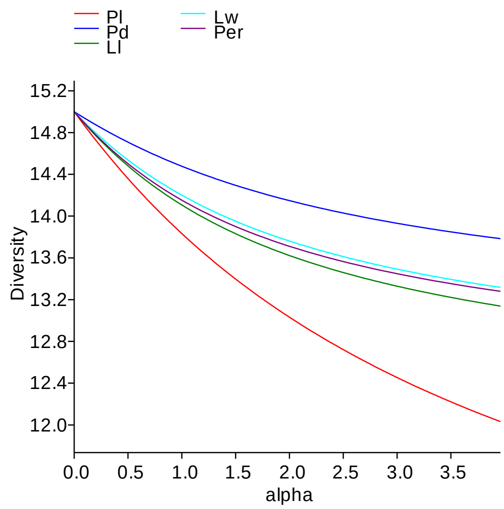

FIGURE 3. Diversity profiles in the case of foliar parameters studied in linden leaves

TABLE 1. The values of the ratio between the foliar parameters studied in the linden leaves

Leaf number	Parameters ratio			
	Pl/Pd	Ll/Lw	Per/Ll	Per/Lw
L1	4.0476	0.9573	3.6752	3.5181
L2	2.1467	0.9689	3.6690	3.5550
L3	1.8733	0.9661	3.7191	3.5930
L4	1.3969	1.0000	3.4299	3.4299
L5	1.3870	1.0049	3.2502	3.2661
L6	1.1160	1.0718	3.1826	3.4111
L7	0.8565	1.0914	3.3222	3.6259
L8	0.9081	1.0710	3.3447	3.5823
L9	1.1557	1.0000	3.3991	3.3991
L10	1.0613	1.0000	3.4994	3.4994
L11	0.9736	0.9831	3.3740	3.3171
L12	0.8889	1.0638	3.1145	3.3133
L13	0.7571	0.9778	3.5010	3.4232
L14	0.6231	0.9467	3.5392	3.3504
L15	0.8750	0.9512	3.2262	3.0688
SE	±0.2210	±0.0124	±0.0476	±0.0382

Analyzing the degree of heterogeneity of the ratio between the studied leaf parameters, a high value of the coefficient of variation was found in the case of the Pl/Pd ratio ($CV_{Pl/Pd}$ = 63.9961). In the case of the other studied reports, lower and closer values of the coefficient of variation were found ($CV_{Ll/Lw}$ = 4.7927; $CV_{Per/Ll}$ = 5.4053; $CV_{Per/Lw}$ = 4.3261).

PCA analysis, in relation to the foliar parameters studied, led to the diagram in Figure 4. PC1 explained 81.459% of variance, and PC2 explained 16.404% of variance. It was found the independent positioning of samples L1, L13, L14 and L15, as initial and final leaves on the length of the shoot (until the time of study). The L2 and L3 samples, and partially L4 and L5, were associated with the Pl parameter (as biplot). The other leaf samples were oriented towards parameters Ll, Lw, Pd and Per (as biplot).

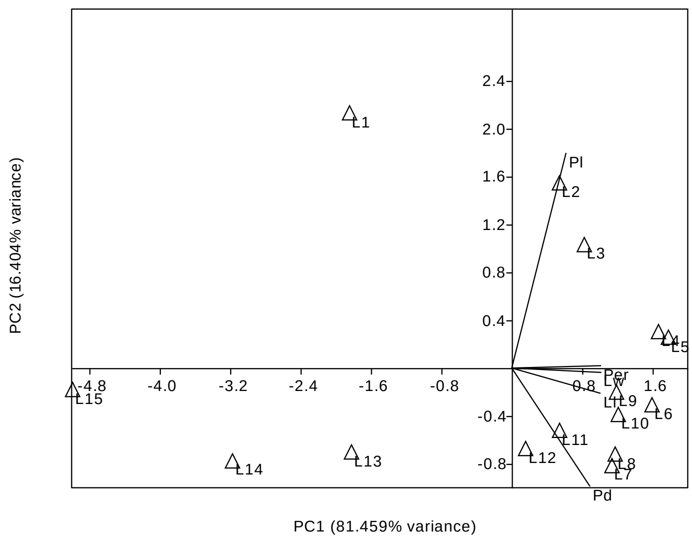

FIGURE 4. PCA diagram with the distribution of leaf cases studied in linden, in relation to the position on the shoot (foliar parameters as biplot)

The cluster analysis of the studied leaf cases led to the dendrogram in figure 5, in which the leaf samples were grouped based on similarity in relation to the values of the studied leaf parameters, in statistical safety conditions, Coph. corr = 0.872. The formation of two distinct clusters was found, with several subclusters each. A C1 cluster comprises the leaves with extreme positions, at the base and top of the shoot at the time of study, L1, L13, L14 and L15, grouped in two sub-clusters. Cluster C2 includes the other leaf samples, grouped in two sub-clusters, based on similarity, in relation to the studied leaf parameters. The highest level of similarity was recorded for L8 and L9 (SDI = 1.3158), followed by L4 and L5 (SDI = 1.3497). The complete set of values of SDI (similarity and distance indices) is presented in table 2.

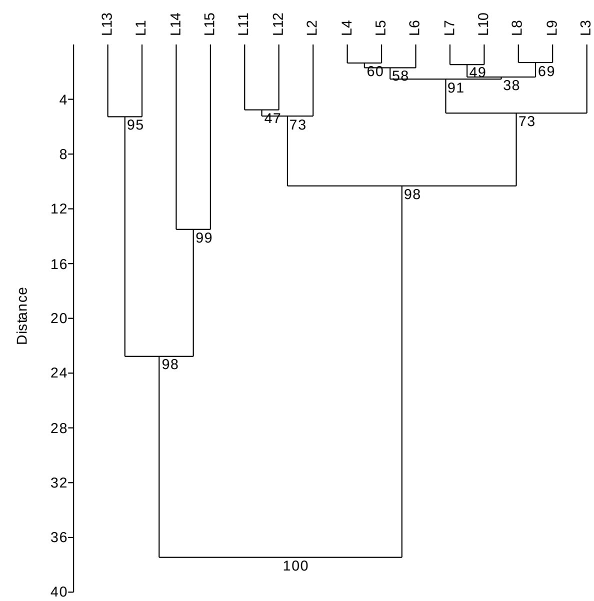

FIGURE 5. The grouping dendrogram of the studied leaves, in relation to the degree of similarity for the studied leaf parameters

The regression analysis evaluated the variation of the leaf perimeters (Per) in relation to the dimensional elements of the leaf (Ll, Lw). Equation (1) was obtained, which described the variation Per in relation to Ll and Lw in statistical safety conditions, $R^2 = 0.999$, $p<0.001$, $F = 3617.452$.

The graphical distribution of the perimeter (Per) in relation to the length and width of the leaves (Ll, Lw) is shown in figure 6 in the form of a 3D model and in figure 7 in the form of isoquants.

From the analysis of equation (1) as well as of the graphical distributions, figures 6 and 7, it was found that the variation of the perimeter (Per) was much more influenced by the length of the leaves (Ll) than by the width of the leaves (Lw).

TABLE 2. SDI values in relation to foliar parameters for linden leaves

	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10	L11	L12	L13	L14	L15
L1		17.392	24.045	28.651	28.619	28.448	28.942	26.923	26.669	28.829	20.265	16.002	5.2726	17.799	30.936
L2	17.392		6.817	11.459	11.584	11.593	12.265	10.243	9.8084	11.912	4.9829	5.4651	20.569	34.818	48.187
L3	24.045	6.817		4.9052	5.3941	5.4754	5.9901	4.2846	3.6603	5.3466	5.3587	9.7442	26.936	41.318	54.731
L4	28.651	11.459	4.9052		1.3497	1.9099	2.8862	3.0174	2.4968	1.8945	8.9301	13.56	31.351	45.774	59.21
L5	28.619	11.584	5.3941	1.3497		1.4839	3.1951	3.2523	2.598	2.534	8.8281	13.367	31.277	45.689	59.109
L6	28.448	11.593	5.4754	1.9099	1.4839		2.0097	2.2258	2.1718	2.0271	8.457	12.956	30.948	45.377	58.806
L7	28.942	12.265	5.9901	2.8862	3.1951	2.0097		2.0927	2.7873	1.4651	8.8893	13.416	31.293	45.735	59.174
L8	26.923	10.243	4.2846	3.0174	3.2523	2.2258	2.0927		1.3158	2.2895	6.8716	11.434	29.305	43.749	57.196
L9	26.669	9.8084	3.6603	2.4968	2.598	2.1718	2.7873	1.3158		2.3191	6.644	11.312	29.182	43.626	57.066
L10	28.829	11.912	5.3466	1.8945	2.534	2.0271	1.4651	2.2895	2.3191		8.8192	13.5	31.311	45.76	59.206
L11	20.265	4.9829	5.3587	8.9301	8.8281	8.457	8.8893	6.8716	6.644	8.8192		4.7686	22.571	37.012	50.455
L12	16.002	5.4651	9.7442	13.56	13.367	12.956	13.416	11.434	11.312	13.5	4.7686		18.06	32.467	45.887
L13	5.2726	20.569	26.936	31.351	31.277	30.948	31.293	29.305	29.182	31.311	22.571	18.06		14.45	27.912
L14	17.799	34.818	41.318	45.774	45.689	45.377	45.735	43.749	43.626	45.76	37.012	32.467	14.45		13.5
L15	30.936	48.187	54.731	59.21	59.109	58.806	59.174	57.196	57.066	59.206	50.455	45.887	27.912	13.5	

$$\text{Per} = ax^2 + by^2 + cx + dy + exy + f \qquad (1)$$

where: Per – leaves perimeter;
x – Ll – leaves length;
y – Lw – leaves width;
a, b, c, d, e, f – coefficients of the equation (1);
a= 4.2533090;
b= 4.6191179;
c= 2.2211942;
d= 0.9834675;
e= -8.8634723;
f= 0

The ratios between foliar parameters express different aspects of leaf typology, proportionality, response to growth conditions and stress factors.

The ratios between some parameters of the geometry of the leaves in plants have been used in studies for the analysis and characterization of the shape of the leaves in different plants species, being considered important and with a high degree of stability (Kalyoncu and Toygar, 2015; Shi *et al*., 2019b; 2021; Liu *et al*., 2021).

The variation of the leaf geometry was studied in relation to the plant species but also to the environmental conditions, and some reports of the leaf parameters were useful in models for describing the leaf geometry (Smith *et al*., 1997; Liu *et al*., 2021; Shi *et al*., 2019b).

In the present study, the values of the ratios calculated based on the analyzed foliar parameters showed high variability in the case of Pl/Pd and Ll/Lw in relation to the position of the leaves on the shoot, respectively low variability in the case of Per/Ll and Per/Lw.

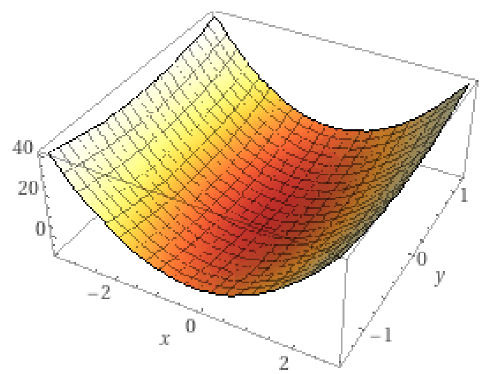

FIGURE 6. 3D model of variation of Per in relation to Ll (x-axis) and Lw (y-axis) for linden leaves

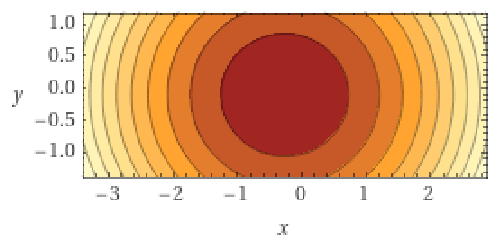

FIGURE 7. Model in the form of isoquants of variation of Per in relation to Ll (x-axis) and Lw (y-axis) for linden leaves

CONCLUSIONS

Mathematical and statistical approach facilitated the highlighting of the degree of variation of the foliar parameters studied in lime, *Tilia tomentosa* Moench. in relation to the position of the leaves on the length of the shoots.

The ratios between the studied parameters registered specific values, in relation to the position of each leaf on the shoot.

The perimeter of the leaves (Per) varied significantly more in relation to the length of the leaves (Ll) than in relation to the width of the leaves (Lw), which denotes the specific contribution of each leaf to the perimeter.

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