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Ginkgo biloba L. leaf area in relation to leaf parameters – Comparative analysis on female and male trees

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Abstract
The study analyzed leaf area in relation to certain leaf parameters in Ginkgo biloba L. leaves, female (F) and male (M) trees. A positive correlation was found, with different levels of intensity, between leaf area (LA) and leaf perimeter (Per), foreground pixels (FP) and fractal dimension (D) of ginkgo leaf shape, respectively. The variation of leaf area (LA) in relation to Per and FP parameters was described by polynomial equations, under conditions of $R^2 = 0.999$, $p<0.001$. The level of fit between measured leaf area (LA) and leaf area predicted based on Per and FP parameters was described by linear equations, with RMSE = 0.08673 (leaves from female tree) and RMSE = 0.09087 (leaves from male tree). Multivariate analysis showed positive action of leaf parameters in Principal Component PC1, with differentiated intensity, r = 0.972 (for LA), r = 0.965 (for FP), r = 0.876 (for D), r = 0.754 (for Per) in the case of samples from female trees, respectively r = 0.987 (for LA), r = 0.982 (for FP), r = 0.982 (for D), r = 0.873 (for Per), samples from male trees.

Keywords: Component loadings; *Ginkgo biloba*; leaf area; leaf parameters; multivariate analysis

Introduction

Leaf area is an important parameter of plant leaves, in relation to the reception of sunlight, specific physiological processes of respiration, photosynthesis, metabolic activities, etc. [46], [37], [21]. Variability in plant leaves has been studied in relation to plant species, environmental conditions, plant age, technological conditions (in the case of cultivated plants), but also in relation to other influencing factors [27], [23], [9], [43], [19]. Leaf shape and leaf area in different plant species were studied and analyzed in relation to radiant energy, photosynthetic efficiency, biomass production, lignocellulose content and certain influencing factors [29], [41], [45], [7].

Plant leaves have been studied as indicators and predictors of climate change, plant growth, and plant adaptations to environmental changes [31], [4], [34]. Foliar parameters are important taxonomic descriptors for plant species studies [33]. Leaf parameters, such as leaf length and width, have been used to estimate leaf area in different plant species [6], [25], [33], [13]. Interdependence relationships were evaluated between the dynamics of foliar parameters and leaf area in different plant species, associated with leaf development, with dynamics during the vegetation period [35], [3].

Leaf shape and the very large variation of this foliar parameter have been analyzed in different plant species, in relation to plant evolution, plant growth and development and in relation to different genetic, biochemical influencing factors, or as a result of the "genotype x environment" interaction [39], [40], [5], [26].

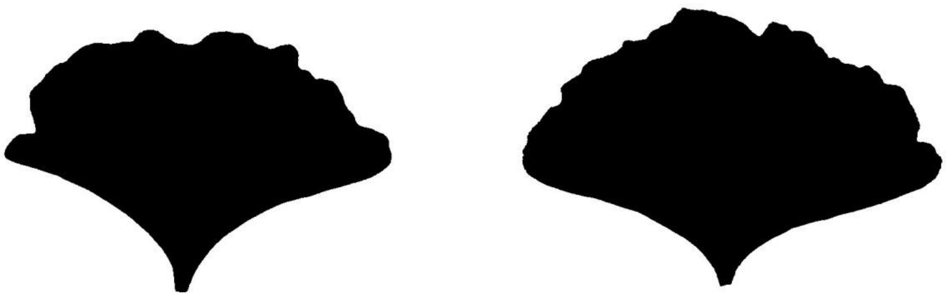
Ginkgo biloba L. is a large deciduous tree [22]. Ginkgo is an ancient species, considered a "living fossil" [14]. Based on its bioactive compounds, ginkgo has multiple uses in the pharmaceutical and medical fields [14], [22], [28]. Ginkgo has also shown interest in the field of ornamental plants, in food, in religion with different cultural values [17], [22], [28]. Ginkgo is a dioecious plant species, with male plants being cultivated ornamentally and female plants for fruit production [10]. Ginkgo leaves have been extensively studied and analyzed in terms of their content of active principles and pharmaceutical and medicinal uses [16], [47]. Ginkgo leaves have been studied from a genetic, morphological, and physiological perspective to understand the formation, evolution, and variability over the long evolution of this species [12], [44], [38], [20].

This study comparatively analyzed leaf area in relation to certain foliar parameters in *Ginkgo biloba* L. leaves from female and male tree specimens, and described through mathematical and statistical analysis the interdependence and variation relationships of LA in relation to the considered foliar parameters.

Material and Method

In accordance with the purpose of the study, *Ginkgo biloba* L. trees, female and male specimens from public spaces in Timisoara, were considered in the study. Leaf samples were randomly collected from the leaf litter deposited on the ground in autumn, Fig. 1. This method of leaf sampling ensured representative specimens for different leaf size categories, and crown position during the growing season. 40 leaf samples were collected from each female tree, respectively from the male tree, Fig. 2.

Figure 1. Carpet of *Ginkgo biloba* L. leaves in autumn [2]



(a) (b)

Figure 2. *Ginkgo biloba* L. leaf specimens, female tree (a) and male tree (b)

The leaves were scanned (HP CM2320fxi MFP), in a 1:1 ratio, to preserve the real proportions of the leaf shape. Image analysis [30] facilitated the obtaining of values for leaf perimeter (Per) and leaf area (LA).

Data series were recorded for each leaf type, female tree, and male tree, respectively. Experimental data processed and analyzed mathematically and statistically, to evaluate the data distribution, the level of interdependence between leaf parameters, the variation of leaf area in relation to leaf parameters, the precision and reliability of leaf area estimation, the position of leaf parameters in relation to the principal components. The reliability of the results was assessed based on established statistical reliability parameters (r, R^2, p, RMSE). PAST software [11], JASP software [15] and Mathematica software [42] were used to analyze the experimental data and generate figures and graphs.

Results and Discussion

Experimental data regarding leaf perimeter (Per) and leaf area (LA), for leaf samples from female (F) and male (M) trees are represented in Fig. 3. The data series showed normal distribution, according to r = 0.983 (Per-F), r = 0.995 (LA-F), r = 0.981 (Per-M), r = 0.995 (LA-M), Fig. 4.

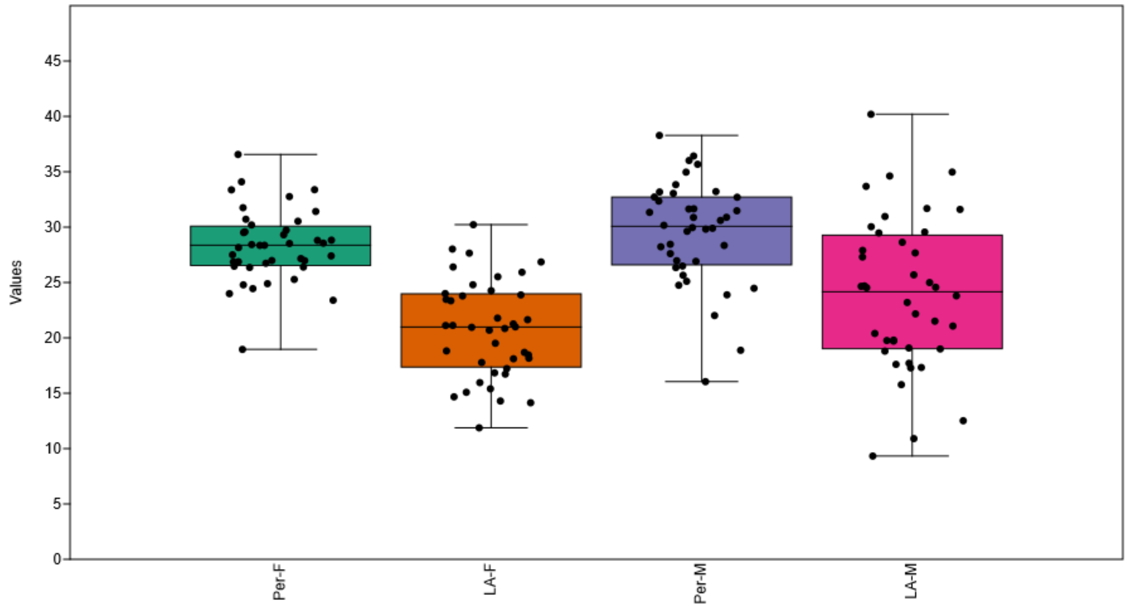


Figure 3. Perimeter and leaf area values in box plot format for ginkgo leaves, female (F) and male (M) trees

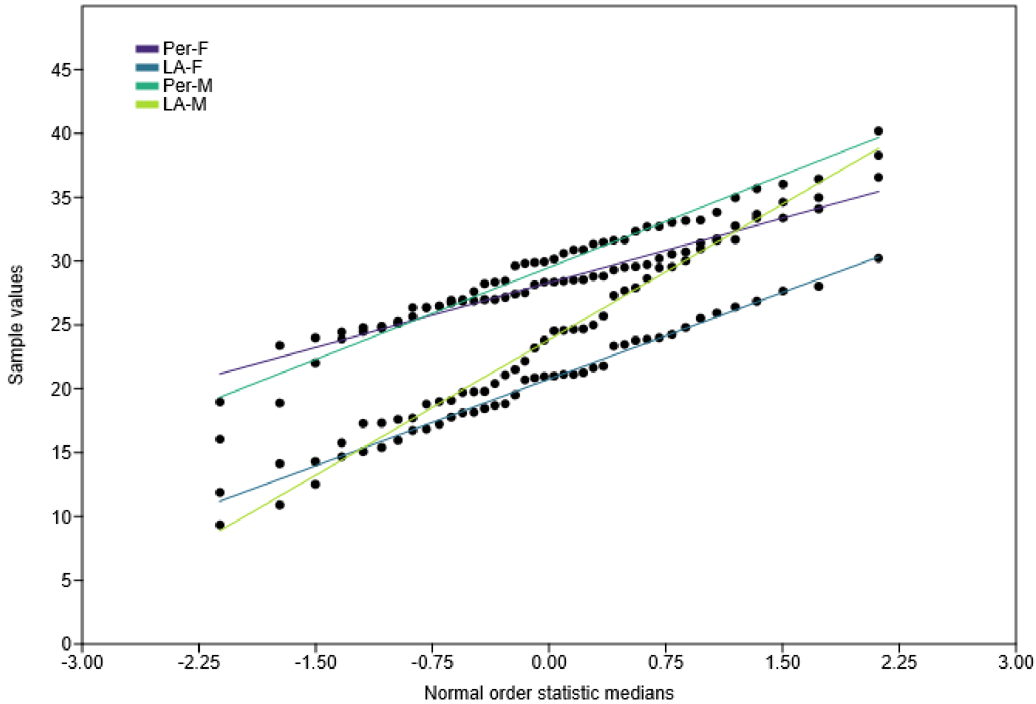


Figure 4. Normal probability plot; Per-F – leaf perimeter from female tree; LA-F – leaf area from female tree; Per-M – leaf perimeter from male tree; LA-M – leaf area from male tree

Correlation analysis (Spearman's rho) showed the level of interdependence between leaf area and foliar parameters. In the case of foliar parameters, in addition to leaf perimeter (Per), foreground pixelx (FP) and fractal dimension (D), obtained in a previous study, were also considered in the correlation analysis [2]. In the case of leaf samples from the female tree, a very strong positive correlation was recorded between LA and FP (r = 0.998***). Positive correlation, moderate in intensity, was recorded between LA and D (r = 0.766***) and between D and FP (r = 0.772). Positive correlation, of weak intensity, was recorded between Per and FP (r = 0.624***), between Per and D (r = 0.547***) and between Per and LA (r = 0.641***), Table 1.

Table 1. Correlation table, ginkgo leaf samples, female tree

	Statistical parameters	Per	FP	D	LA
Per	Spearman's rho	—			
	p-value	—			
FP	Spearman's rho	0.624***	—		
	p-value	< .001	—		
D	Spearman's rho	0.547***	0.772***	—	
	p-value	< .001	< .001	—	
LA	Spearman's rho	0.641***	0.998***	0.766***	—
	p-value	< .001	< .001	< .001	—

In the case of leaf samples from male trees, a positive correlation of very strong intensity was recorded between LA and FP (r = 0.999***), between LA and D (r = 0.991), and between D and FP (r = 0.992). Positive correlation, moderate in intensity, was recorded between LA and Per (r = 0.767***), between D and FP (r = 0.758) and between FP and Per (r = 0.755), Table 2.

Table 2. Correlation table, male ginkgo tree leaf samples

	Statistical parameters	Per	FP	D	LA
Per	Spearman's rho	—			
	p-value	—			
FP	Spearman's rho	0.755***	—		
	p-value	< .001	—		
D	Spearman's rho	0.758***	0.992***	—	
	p-value	< .001	< .001	—	
LA	Spearman's rho	0.767***	0.999***	0.991***	—
	p-value	< .001	< .001	< .001	—

Regression analysis described the variation of LA in relation to leaf perimeter (Per) and foreground pixels (FP), related to leaf shape.

In the case of leaves from the female tree (F), the variation in leaf area (LA) in relation to Per and FP was described by Equation (1), with R^2 = 0.999, F = 16939.2117, p<0.001, with the graphical representation in Fig. 5.

$$\mathrm{LA}_{(F)} = ax^2 + by^2 + cx + dy + exy + f \quad (1)$$

where: $\mathrm{LA}_{(F)}$ – leaf area for ginkgo leaves, female tree; x – leaf perimeter (Per), y – forreground pixels (FP); a, b, c, d, e, f – the coefficients of equation (1)

a = -0.0014337
b = -2.8E-11
c = 0.086518
d = 0.000513
e = 6.07E-07
f = -0.441493

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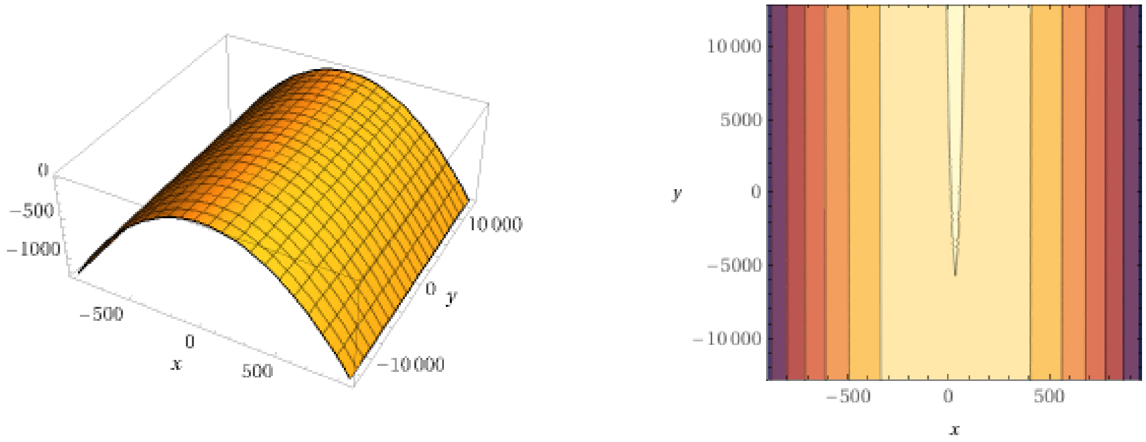


(a) (b)

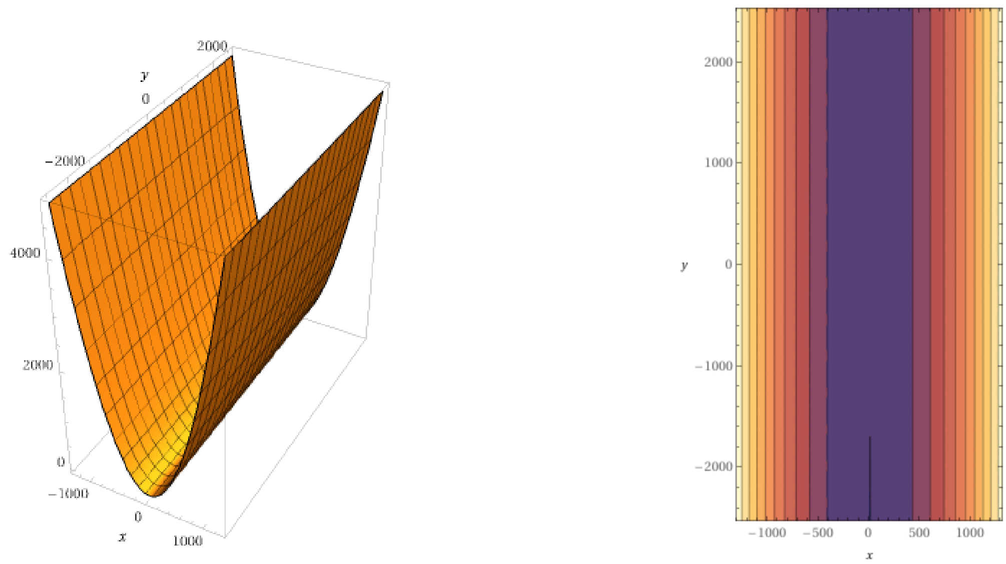
Figure 5. Graphical distribution of LA in relation to Per (x – axis) and FP (y – axis) in ginkgo leaves, female tree; (a) – 3D model, (b) model in isoquant format

In the case of leaves from the male tree (M), the variation in leaf area (LA) in relation to Per and FP was described by Equation (2), with R^2 = 0.999, F = 37783.6764, p<0.001, with graphical representation in Fig. 6.

$$\mathrm{LA}_{(M)} = ax^2 + by^2 + cx + dy + exy + f \quad (2)$$

where: $\mathrm{LA}_{(M)}$ – leaf area for ginkgo leaves, male tree; *x* – leaf perimeter (Per), *y* – forreground pixels (FP); a, b, c, d, e, f – the coefficients of equation (2)

a = 0.002816
b = -8.99E-12
c = -1.056902
d = 0.000170
e = -3.2E-07
f = 0.575619



(a) (b)

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

The RMSE statistical parameter, Equation (3), was used to verify the accuracy of estimating LA based on leaf parameters (Per, FP) for the two categories of leaf samples, from female trees and from male trees, respectively.

$$\mathrm{RMSE} = \sqrt{\frac{1}{n}\sum_{j=1}^{n}\left(y_j - \hat{y}_j\right)^2} \tag{3}$$

In the case of leaf area (LA) for leaf samples from female trees, the estimation accuracy was confirmed at RMSE = 0.08673. The level of fit between the scanned leaf area (LA) and the leaf area estimated based on the Per and FP parameters was described by linear Equation (4), with graphical representation in Fig. 7(a).

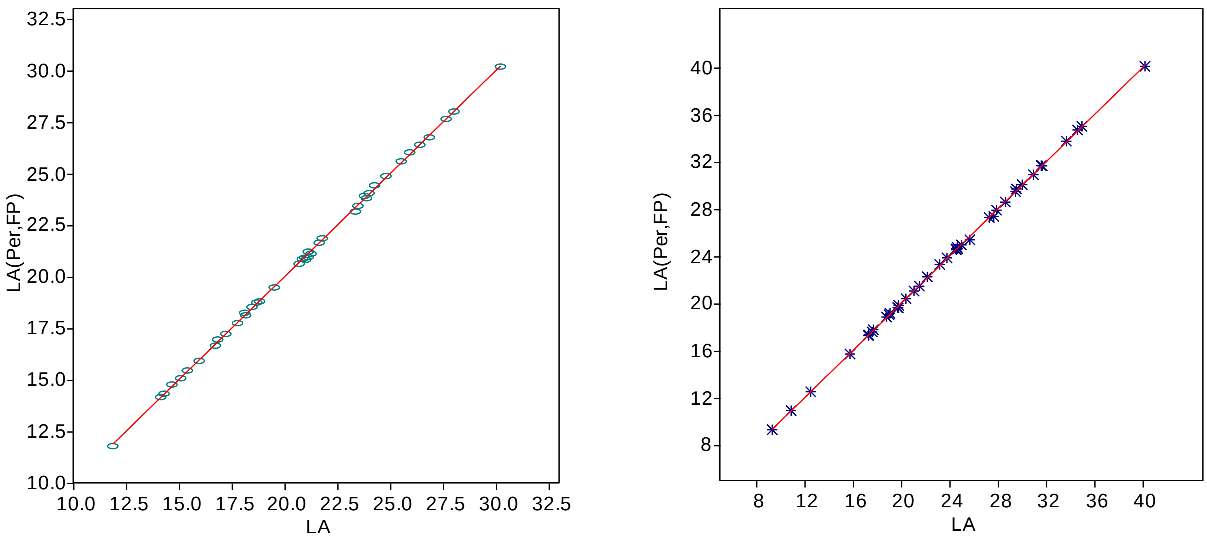
$$LA_{(Per,FP)} = 0.9996\,x + 0.008331 \tag{4}$$

where: $LA_{(Per,FP)}$ – leaf area predicted based on Per and FP parameters for leaves from female tree; x – measured leaf area by scanning from female tree

In the case of leaf area for leaf samples from male trees, the estimation accuracy was confirmed at RMSE = 0.09087. The level of fit between the scanned leaf area (LA) and the leaf area estimated based on the Per and FP parameters was described by the linear Equation (5), with graphical representation in Fig. 7(b).

$$LA_{(Per,FP)} = 0.9998\,x + 0.004293 \tag{5}$$

where: $LA_{(Per,FP)}$ – leaf area predicted based on Per and FP parameters for leaves from male tree; x – measured leaf area by scanning for leaves from male tree



(a) (b)

Figure 7. Fitting line between measured leaf area and predicted leaf area based on leaf parameters (Per, FP); (a) female tree leaf samples; (b) male tree leaf samples

Multivariate analysis (PCA) was used to evaluate the position of leaf parameters (as factors) in relation to the Principal Components. Chi-squared Test confirmed the reliability of the analysis and the recorded results (p<0.001), Table 3.

Leaf parameters (as factors) were distributed in PC1 for both categories of leaf samples (female tree, male tree), Table 4, with component characteristics presented in Table 5.

Table 3. Chi-squared Test results, parameters in ginkgo leaf samples

Leaf samples	Model	Statistical parameters		
		Value	df	p
Female tree (F)	Model (F)	174.633	2	< .001
Male tree (M)	Model (M)	173.807	2	< .001

Table 4. Component Loadings, sample parameters ginkgo leaves

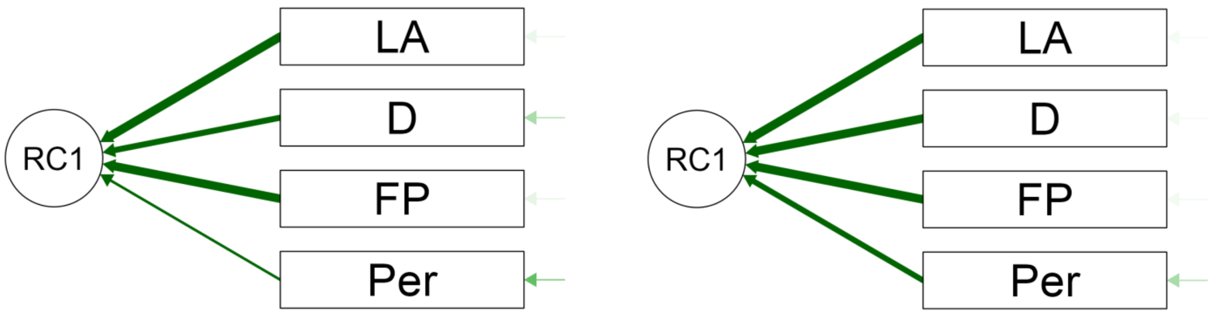
Leaf samples type					
Female			Male		
Leaf parameters	PC1	Uniqueness	Leaf parameters	PC1	Uniqueness
LA	0.972	0.055	LA	0.987	0.027
FP	0.965	0.070	FP	0.982	0.035
D	0.876	0.233	D	0.982	0.036
Per	0.754	0.432	Per	0.873	0.238

Table 5. Component Characteristics

Samples	Components	Unrotated solution			Rotated solution		
		Eigenvalue	Proportion var.	Cumulative	SumSq. Loadings	Proportion var.	Cumulative
Female	Component 1	3.211	0.803	0.803	3.211	0.803	0.803
Male	Component 1	3.664	0.916	0.916	3.664	0.916	0.916

In the case of leaf samples from female trees, leaf parameters (as factors) were loaded in PC1, with positive action, of very strong intensity, r = 0.972 (for LA), r = 0.965 (for FP), of strong intensity, r = 0.876 (for D), and of moderate intensity, r = 0.754 (for Per), Table 4, Fig. 8(a).

In the case of leaf samples from male trees, leaf parameters (as factors) were loaded into PC1, with very strong action, r = 0.987 (for LA), r = 0.982 (for FP), r = 0.982 (for D), and with strong action, r = 0.873 (for Per), Table 4, Fig. 8(b).



(a) (b)

Figure 8. Graphical representation of the factor loadings in the principal component PC1; (a) leaf parameters from the female tree; (b) leaf parameters from the male tree

The statistical analysis showed the normal distribution of the data series for foliar parameters considered in ginkgo leaves, samples from female and male trees. The applied statistical test showed that there were no values in the “outliers” position, according to p = 0.104 (Per), p = 1 (FP), p = 1 (D), p = 1 (LA) for the data series from female trees, respectively p = 0.0925 (Per), p = 0.592 (FP), p = 0.1179 (D), p = 0.5536 (LA) for the data series from male trees.

Interdependence and correlations between foliar parameters were evaluated in different plant species, in relation to the position of the leaves on the plant, the age of the leaves, the response of the plants to environmental conditions, ecosystem aspects, etc. [18], [1], [3], [4].

Positive correlations were recorded between foliar parameters, but of different levels of intensity. In the leaf samples from the female tree, a very strong correlation was recorded between LA and FP (r = 0.998***), and the other correlations were of moderate intensity (two correlations) and weak intensity (three correlations). In the case of leaf samples from the male tree, three very strong correlations were recorded between D and FP (r = 0.992***), between LA and FP (r = 0.999***) and between LA and D (r = 0.991***) and three correlations of moderate intensity.

Various mathematical models have been reported to describe the leaf blade in plants, to predict leaf area variation in relation to leaf size, or to predict leaf area [8], [6], [3].

In the present study, quadratic polynomial models described the variation of LA in relation to foliar parameters (Per, FP) with statistical certainty ($p<0.001$). Linear regression analysis showed a very high level of fit between measured and predicted LA values.

Multivariate analysis highlighted the correlation of some foliar parameters at the plant leaf level, and facilitated the classification of plant genotypes or plant samples in relation to influencing factors, or the description of foliar aspects at the plant and agricultural crop level [36], [24], [32].

In the analysis of ginkgo leaf samples, multivariate analysis showed the positioning of leaf parameters in relation to the principal components, the positive action of the parameters in PC1 and the variable level of action intensity.

Conclusions

Leaf samples taken from Ginkgo biloba, female and male trees, were representative, expressed variability in size and positioning in the crown, and the data series for leaf parameters presented a normal distribution, without outlier values.

Positive correlation was recorded between foliar parameters in all leaf samples, but several correlations with very strong intensity were recorded between parameters in leaf samples from male trees.

Polynomial mathematical models and graphical models (3D, isoquants) described the variation of leaf area (LA) in relation to leaf perimeter (Per) and foreground pixels (PF), with statistical certainty ($p<0.001$). Linear regression analysis confirmed a very high level of fit between predicted and measured leaf area.

Foliar parameters considered in the study were loaded into the Principal Component PC1, with positive action, and variable intensity levels. In the leaf samples from the male tree, very high intensity of action of the foliar parameters in PC1 was recorded for three parameters (LA, FP, D) and strong action in the case of the Per parameter. In the case of leaf samples from the female tree, very high intensity of action of the foliar parameters in PC1 was recorded for two parameters (LA, FP), strong action in the case of the D parameter and moderate action in the case of the Per parameter.

The leaf parameters considered can represent indicators of differentiation of gonkgo specimens, of the vegetation status of plants in relation to vegetation factors, and of response to stress factors.

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