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FRACTAL ANALYSIS IN THE COMPARATIVE STUDY OF *GINKGO BILOBA* L. TREES

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Abstract

The study aimed to detect female and male plants of the species Ginkgo biloba L. based on the fractal properties of leaf geometry. Leaf samples (40 leaves) were taken from female and male tree specimens, from different locations (Timisoara and Deva). To obtain representative samples, by leaf size category, samples were taken randomly, in autumn after leaf fall, on the leaf carpet on the ground, under the crown projection. The box-counting method was used to analyze the leaf geometry and obtain the values of fractal dimensions (D). The fractal analysis led to the fractal dimensions (D), denoted D(F1), D(F2) for female tree specimens and D(M1), D(M2) for male tree specimens. The mean values of the fractal dimensions were D(F1) =1.6737±0.0069, D(F2) =1.5842±0.0125, D(M1) =1.4947±0.0107, D(M2) =1.5146±0.0051. Each sample D(F1), D(F2) was analyzed compared to each sample D(M1), D(M2). Comparative analysis of fractal dimension values (means, medians) from female and male specimens led to statistically significant differences (p<0.001). Fractal analysis was a reliable method for detecting the two categories of trees, female and male, under the study conditions.

Key words: *box-counting, comparative analysis, fractal dimension, ginkgo leaves, hierarchical clustering.*

INTRODUCTION

Ginkgo biloba L. (Ginkgoaceae, Ginkgoales), is a dioecious, deciduous tree species native to southeastern China (Cheng & Fu, 1978; Lin et al., 2022). Ginkgo has high ecological plasticity, with adaptability to very varied climatic conditions (Lin et al., 2022).
Ginkgo has high socio-cultural value for several nations, along with important ecological and economic benefits (Crane, 2019; Lin et al., 2022; Lee et al., 2023).
Ginkgo has been referred to as a plant with numerous "compounds with unique structures", which resulted from its distinct biological characteristics (Liu et al., 2022). *Ginkgo biloba* contains a series of active principles of importance for the pharmaceutical, medical, cosmetic, and food industries (Liu et al., 2022; Noor-E-Tabassum et al., 2022; Akaberi et al., 2023). Favorable effects of some preparations and products containing active ginkgo principles have been recorded in the pharmacological and medical fields (Akaberi et al., 2023).
Ginkgo biloba has been found to be a species with very good adaptability to the urban environment, to stress factors specific to the urban environment, and ginkgo trees are present in various parks and green spaces in the urban environment (Cui et al., 2022; Lin et al., 2022; Kisvarga et al., 2024).
Fossilized ginkgo leaves have been studied in relation to factors and conditions specific to the geological eras in which they formed and grew (Sun et al., 2003). The comparative analysis of some ginkgo genotypes was made based on foliar parameters (Klimko et al., 2015).
Different morphological types of leaves have been identified in ginkgo, as a different expression of the genes involved and the molecular mechanisms (Tang et al., 2022).
Different leaf types and leaf modifications have been identified and reported in ginkgo, with variable frequency, higher in the normal form (Li et al., 2024). It was confirmed that morphological differentiation of leaves in ginkgo occurred at the level of leaf primordia, through gene expression and specific molecular processes (Li et al., 2024).
Various methods of analysis, microscopic, geometric, topological, have been used to analyze and study ginkgo leaves (Carvalho et al., 2017; Hang et al., 2021). Based on the

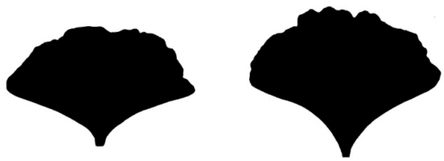
results, Hang et al. (2021) identified certain distinctive elements, specific to the studied leaves, which led to the differentiation of ginkgo leaf shape.
Early differentiation of female and male specimens in ginkgo seedlings is important for the selection of seedlings in relation to the planting destination, the purpose for which they are cultivated, and different "ginkgo industries" (Gao et al., 2024). Some differences in ginkgo seedlings were recorded, for their growth capacity and rate (higher in male specimens), and for their content of bioactive compounds (higher in female specimens) (Gao et al., 2024). According to the authors, the results presented interest for a base of indicators in the early selection of female and male specimens in ginkgo (Gao et al., 2024).
The male forms of ginkgo presented certain advantages for practice, and were of interest for landscaping, and the female forms for fruit production (Fu et al., 2021; Li et al., 2023).
Electrochemical techniques (peroxidase content detection) and molecular techniques (male-specific SCAR gene) were used to identify and select female and male ginkgo forms (Fu et al., 2021; Lee et al., 2023).
This research attempted to differentiate female and male forms in mature ginkgo trees, based on leaf geometry expressed in fractal dimension (D).

MATERIALS AND METHODS

For the comparative analysis of female and male specimens of *Ginkgo biloba* L. trees, the leaves were used as biological material. Leaf samples were taken in autumn from the carpet of fallen leaves on the ground (Figure 1).

Figure 1. Leaf mat, *Ginkgo biloba* L. species; base for collecting leaf samples at variable distances from the stem

On the leaf mat, on the ground, the leaves came from different areas of the crown, and in this way a much more homogeneous sampling base was ensured. Leaf samples, of variable sizes, were taken randomly, at different distances from the tree trunk. 40 leaves were taken for each sample.
Two female specimens of ginkgo trees from Timisoara (F1) and Deva (F2), and two male specimens of ginkgo trees from Timisoara (M1, M2), were considered. In all cases, the samples were collected from mature trees.
On the leaf litter, the leaves came from different areas of the tree crown. Thus, the sampling base had a high degree of homogeneity. Leaf samples of different sizes were taken from several points on the leaf litter below the crown projection.
Each leaf was scanned at 1:1 scale (HP, CM2320fxi MFP, fixed scanner). The digital images, binarized (Figure 2), were analyzed to obtain the fractal geometry parameters of the leaves. The foreground pixels (FP) values and the fractal dimension (D) were recorded. Box-counting analysis was used (Voss, 1985), and ImageJ (Rasband, 1997).



(a) (b)

Figure 2. Examples of scanned leaves, *Ginkgo biloba* L. species; (a) female specimen leaf, (b) male specimen leaf; images in binarized format

The overall evaluation of the data series was done through descriptive statistical analysis, and the reliability of the data was done through the Anova test. The interdependence relationship between fractal dimensions (D) and foreground pixels (FP) was evaluated by regression analysis. The comparison between leaf samples from female specimens, D(F1), D(F2) and male specimens (D(M1), D(M2) was made on the basis of fractal dimension, mean values (t-test), and median values (Wilcoxon test). Established statistical confidence parameters were used to assess the reliability of the results (p, and RMSE). The mathematical analysis on the fractal parameters in ginkgo leaves was made in EXCEL, and in the PAST software (Hammer et al., 2001).

RESULTS AND DISCUSSIONS

Ginkgo leaves, in total 160 leaves, collected from the two categories, in two repetition foe each category (40 from each category), two female specimens (F1, F2) and two male categories (M1, M2) were analyzed to describe the fractal geometry of the leaves. Values were obtained for the foreground pixels (FP) parameter and for the fractal dimension (D) for each sample category.

Descriptive statistical analysis generated the synthetic values in Table 1. According to the Anova Test (95% confidence), the results statistical safety was confirmed (Table 2). The presence of variance was also confirmed, as shown in Table 2.

Table 1. Descriptive statistical analysis report, *Ginkgo biloba* leaf samples, female and male specimens

Statistical parameters	Fractals parameters							
	Female tree specimens				Male tree specimens			
	F1 samples		F2 samples		M1 samples		M2 samples	
	FP(F1)	D(F1)	FP(F2)	D(F2)	FP(M1)	D(M1)	FP(M2)	D(M2)
N	40	40	40	40	40	40	40	40
Min	66603	1.5839	95033	1.4309	53769	1.3270	97666	1.4279
Max	174685	1.7445	396132	1.7601	234369	1.5983	225410	1.5707
Sum	4783749	66.9499	8666929	63.3693	5540525	59.4999	6321522	60.5840
Mean	119593.7	1.6737	216673.2	1.5842	138513.1	1.4875	158038	1.5146
Std. error	4091.015	0.0069	11733.08	0.0125	6422.454	0.0090	4650.453	0.0051
Variance	6.69E+08	0.001926	5.51E+09	0.006237	1.65E+09	0.00321	8.65E+08	0.001046
Stand. dev	25873.85	0.043884	74206.51	0.078976	40619.17	0.056658	29412.05	0.032339
Median	120178	1.6738	210613	1.5828	139661	1.4979	155011	1.5154
25 percentile	98701.75	1.64825	154177	1.5228	111353.3	1.4500	138858	1.49515
75 percentile	140103.5	1.711325	253433.3	1.625175	171215.3	1.5330	182144.8	1.539425
Skewness	0.0567	-0.4375	0.5474	0.4294	0.1142	-0.6809	0.1379	-0.5522
Kurtosis	-0.6919	-0.4640	-0.0317	-0.1727	-0.2385	0.6367	-0.1924	0.2047
Geom. mean	116769.10	1.67	204307.50	1.58	132208.30	1.49	155312.20	1.51
Coeff. var	21.63	2.62	34.25	4.99	29.33	3.81	18.61	2.14

Table 2. Summary of the Anova Test

Source of Variation	SS	df	MS	F	P value	F crit
Between Groups	2.21E+12	7	3.16E+11	291.1555	1E-132	2.0390
Within Groups	3.39E+11	312	1.09E+09			
Total	2.55E+12	319				

The series of fractal dimension data, D(F1), D(F2), D(M1), and D(M2) presented a normal distribution, with r = 0.9804 (D(M1) to r = 0.9858 (D(M2) (Figure 3).

The FP parameter showed low variability in the FP(M2) data series, CV = 18.61, medium variability in the FP(F1) data series, CV = 21.63, and in the FP(M1) data series, CV = 29.33. High variability was recorded in the FP(F2) data series, CV = 34.25.

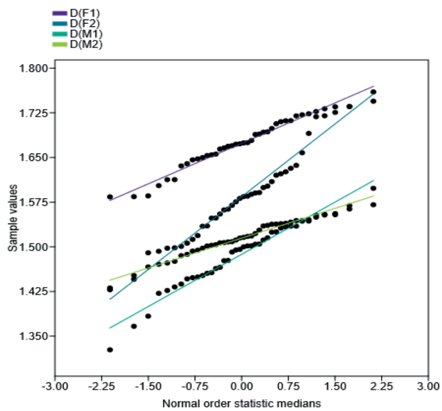


Figure 3. Distribution of D values

In the case of fractal dimension (D), low variability was recorded in all data series, CV =

2.14 (D(M2) to CV = 4.99 (D(F2).
The variation of fractal dimension D was analyzed in relation to the values of the FP parameter, for each leaf sample.
The series of D(F1) values varied in relation to FP(F1) according to equation (1), R^2 = 0.635, F = 34.87, p<0.001 (Figure 4).
The series of D(F2) values varied in relation to FP(F2) according to equation (2), R^2 = 0.562, F = 23.871, p<0.001.
The series of D(M1) values varied in relation to FP(M1) according to equation (3), R^2 = 0.986, F = 1327.3, p<0.001 (Figure 5).
The series of D(M2) values varied in relation to FP(M2) according to equation (4), R^2 = 0.973, F = 660.23, p<0.001.

$$D(F1) = -6.61E-12x^2 + 2.948E-06x + 1.42 \quad (1)$$
where: x – FP(F1)

$$D(F2) = -2.308E-12x^2 + 1.859E-06x + 1.302 \quad (2)$$
where: x – FP(F2)

$$D(M1) = -4.971E-12x^2 + 2.761E-06x + 1.208 \quad (3)$$
where: x – FP(M1)

$$D(M2) = -4.933E-12x^2 + 2.647E-06x + 1.224 \quad (4)$$
where: x – FP(M2)

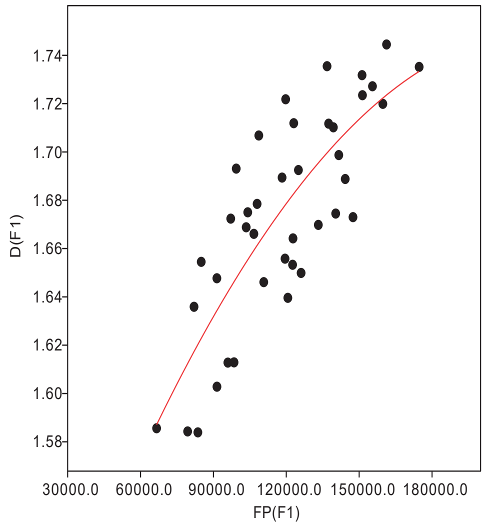


Figure 4. Variation of D(F1) in relation to FP(F1)

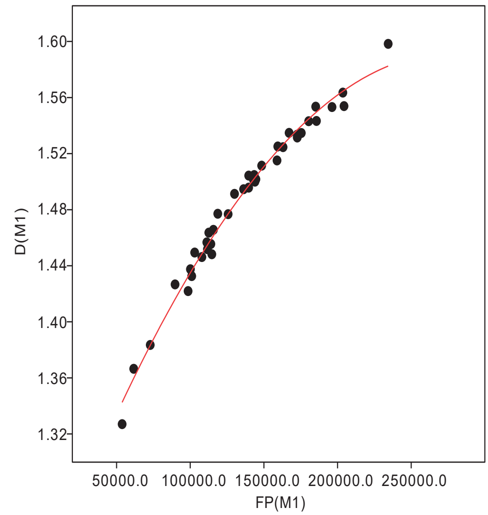


Figure 5. Variation of D(M1) in relation to FP(M1)

From the analysis of the values of the regression coefficient (R^2), the parameter F, equations (1) – (4), as well as the graphical distribution (Figures 4 and 5), a much closer (stronger) relationship was observed between the parameter D and FP in the case of leaf samples from male trees (DM), compared to the situation of leaf samples from female trees (DF).
RMSE values confirmed the high reliability of the D and FP relationship in the case of leaf samples from ginkgo male specimens, compared to female ones; RMSE = 0.0255 for D(F1)–FP(F1) relationship, RMSE = 0.0516 for D(F2)–FP(F2) relationship, RMSE = 0.0066 for D(M1)–FP(M1) relationship, and RMSE = 0.0053 for D(M2)–FP(M2) relationship.
Comparative analysis of leaf samples from female Ginkgo tree specimens was done, based on fractal dimensions (D), in relation to each leaf sample from male tree specimens.
From the analysis of the series of fractal dimensions D(F1), associated with leaves from female tree specimens, with the fractal dimensions from male specimens, D(M1), and D(M2), the statistical values of the applied tests resulted (Table 3).
The mean value, and the median, in the case of D(F1) were higher compared to the mean and median values of D(M1), and D(M2), p<0.001 (Table 3).

Table 3. Comparative analysis results, D(F1) samples compared to D(M1), D(M2) leaf samples

Statistical parameters	Test values compared to D(M)	
	D(M1)	D(M2)
Given mean: D(F1)	1.6737	1.6737
Sample mean: D(M)	1.4875	1.5146
95% conf. interval:	(1.4694 1.5056)	(1.5043 1.5249)
Difference:	0.1862	0.1591
95% conf. interval:	(0.16808 0.20432)	(0.14876 0.16944)
t :	-20.785	-31.115
p (same mean):	1.05E-22	3.78E-29
Significance of mean differences	***	***
Given median: D(F1)	1.6738	1.6738
Sample median: D(M)	1.4979	1.5153
W :	820	820
Normal appr. z :	5.5109	5.5109
p (same median):	3.57E-08	3.57E-08
Significance of median differences	***	***

In the case of fractal dimensions D(F2), associated with leaf samples from female tree specimens, analyzed in comparison with fractal dimensions from male specimens, D(M1), and D(M2), the values of the applied tests resulted (Table 4). The mean value and median in the case of D(F2) were higher compared to the mean and median values of D(M1), and D(M2), $p<0.001$.

Table 4. Comparative analysis results, D(F2) samples compared to D(M1), D(M2) leaf samples

Statistical parameters	Test values compared to D(M)	
	D(M1)	D(M2)
Given mean: D(F2)	1.5842	1.5842
Sample mean: (D(M)	1.4875	1.5146
95% conf. interval:	(1.4694 1.5056)	(1.5043 1.5249)
Difference:	0.096702	0.0696
95% conf. interval:	(0.078582 0.11482)	(0.059257 0.079943)
t :	-10.795	-13.612
p (same mean):	2.81E-13	2.13E-16
Means are significantly different	***	***
Given median: D(F2)	1.5828	1.5828
Sample median: D(M)	1.4979	1.5153
W :	819	820
Normal appr. z :	5.4975	5.5109
p (same median):	3.85E-08	3.57E-08
Medians are significantly different	***	***

The mean and median values of fractal dimensions (D), and the differences calculated for leaf samples from female specimens D(F1), D(F2), compared to male specimens D(M1), D(M2), are presented in Figure 6 for the mean values, and in Figure 7 for the median values.

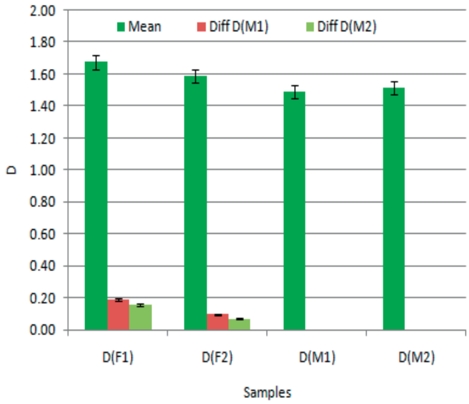


Figure 5. The differences calculated in the case of mean values of fractal dimensions

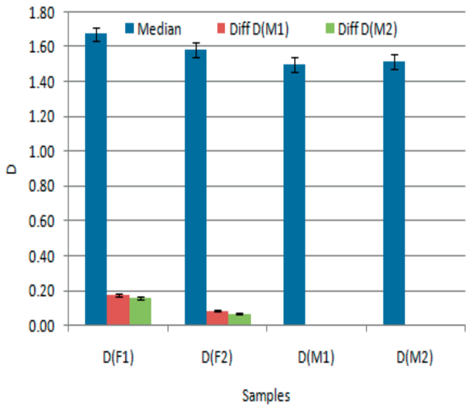


Figure 6. The differences calculated in the case of median values of fractal dimensions

The degree of similarity between the fractal dimensions of male specimens, D(M1), D(M2) was at the level of SDI = 0.4014. The similarity level between D(M1) and D(F1) was SDI = 1.2832, and between D(M1) and D(F2) was SDI = 0.7900. The level of similarity between D(M2) and D(F1) was SDI = 1.0576, and between D(M2) and D(F2) was SDI = 0.6907. In all cases, the level of similarity, based on D values, was lowest between male forms, and

highest in the comparative relationship between male and female forms.
The four leaf types were positioned in distinct clusters, D(F1) with D(F2), and D(M1) with D(M2) within the cluster diagram (Coph.corr. = 0.683), hierarchical clustering (Figure 7).

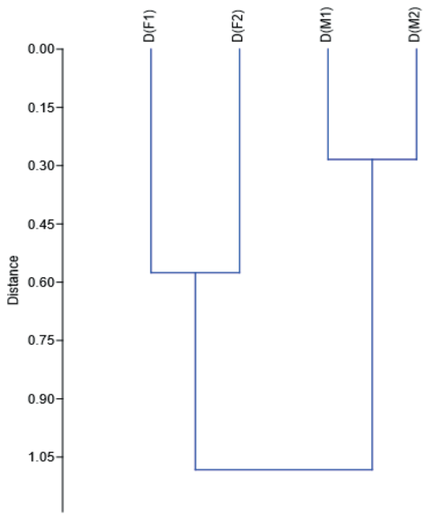


Figure 7. Cluster arrangement of fractal dimensions in female and male forms, ginkgo leaves

Studies on the leaves of trees and shrubs have analyzed anatomical, morphological, and physiological aspects, as well as comparative analyses, classification methods, and responses to vegetation and environmental stress factors, etc. (H'ng & Loh, 2018; Delian and Săvulescu, 2023; Delian et al., 2024; Săvulescu et al., 2024).
Leaves from different ginkgo genotypes were studied and differentiated based on morphological parameters, and obvious differentiations were recorded based on leaf and petiole sizes (Klimko et al., 2015). Leaf and petiole dimensions, as well as certain elements at the "adaxial/abaxial" level, were considered highly specific parameters, useful for differentiating ginkgo leaves from the tested genotypes (Klimko et al., 2015).
Through molecular tests of a collection of 42 ginkgo samples, female and male specimens were identified in a 1:1 ratio. Differentiating female and male forms of ginkgo was of interest for the optimal use of seedlings (Li et al., 2023).
Multivariate analysis (PCA) provided a certain level of separation between female and male ginkgo forms based on tree growth parameters, but there were no differentiations based on wood properties (Li et al., 2023).
The differentiation of leaf shape in ginkgo was recorded based on specific elements, through geometric and topological analysis methods (Hang et al., 2021). Distinctly different values of fractal dimension have been reported in ginkgo in relation to leaf shape (Vlcek & Cheung, 1986). Fractal analysis was applied to highlight certain similarities and specific fractal characteristics with ginkgo leaves (Malischewsky, 2014).
Fractal analysis has captured significant differences in leaf shape in studies of different species of tree plants, or cultivated plants (Vlcek & Cheung, 1986; Sala et al., 2017; Agapie et al., 2020).
To characterize ginkgo trees, in relation to other species, based on laser images, fractal analysis provided significant differentiations (Zhang et al., 2021).
In the case of this research, on leaf samples from mature ginkgo trees, it was possible to differentiate female and male forms, based on the fractal dimension of the leaf geometry.
There is possible variability in the shape of ginkgo leaves in relation to the age of the trees, and this approach, to the fractal geometry of the leaves, requires investigation in comparative studies for clarification, and continued research.

CONCLUSIONS

Fractal analysis described the geometry of ginkgo leaves through fractal dimension values, denoted D(F) for female specimens, and D(M) for male specimens.
The average values of fractal dimensions in female ginkgo leaves, D(F), were higher compared to the fractal dimensions in male leaves, D(M).
The comparative analysis between the two categories of samples, female specimens D(F1), D(F2), and male specimens D(M1), D(M2), led to significant differences for the mean and median values.
A stronger relationship was recorded between the fractal dimension D, and the FP parameter in the case of leaves in male specimens,

compared to female specimens (R^2 = 0.973 in the relationship D(M1) with (FP(M1); R^2 = 0.635 in the relationship D(F1) with FP(F1)).

The results recorded were eloquent for leaf samples from mature ginkgo trees, but require verification in young specimens, where the differentiation of female and male forms is of interest for the differentiated use of seedlings.

In the case of ginkgo seedlings, the accuracy of the method may be lower, due to the lower number of leaves. It is recommended to test this method together with other more accurate but also more expensive methods (e.g. molecular, genetic), for verification and calibration, with the aim of further promotion on a larger scale.

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