

CHARACTERIZATION OF LEAF GEOMETRY AT *Datura stramonium* L. BY IMAGING ANALYSIS

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Abstract

Datura stramonium L. is a species with different active principles, it has toxicity, but it has found certain uses in the pharmaceutical, medicinal, or food field. In certain pedoclimatic conditions it is present in agricultural crops, as a weed. The study analyzed the geometry of the leaves in the species *Datura stramonium* L. A set of leaves was taken randomly from different specimens of mature plants. The leaves were scanned and analyzed in terms of dimensional parameters (L - length, w - width, Per - perimeter), leaf surface (SLA - scanned leaf area; MLA - measured leaf area), leaf geometry (D - fractal dimension). The experimental data set presented statistical safety, according to the ANOVA test ($F > F_{crit}$, $p < 0.001$, for $\alpha = 0.001$). Very strong and strong correlations were recorded between foliar parameters and SLA, MLA and D respectively (eg $r = 0.930$ between MLA and L; $r = 0.969$ between MLA and w; $r = 0.946$ between Per and SLA; $r = 0.933$ between D and SLA). Polynomial equations described the interdependence relations between foliar parameters and SLA, MLA and D, in statistical safety conditions ($p < 0.001$).

Key words: *Datura stramonium*, fractal geometry, imaging, leaves.

INTRODUCTION

Datura stramonium L. is an annual species in the Solanaceae family. The species is widespread on the globe, in different ecoclimates (Akbar, 2020). The whole plant (root, stem, leaves, flowers) is toxic, with variations in the level of toxicity depending on the organs of the plant (Trancă et al., 2017).

The herb of *Datura stramonium* L. presents a series of active principles with potential for use in pharmacy and medicine (Li et al., 2012; Soni et al., 2012; Aboluwodi et al., 2017).

The content of active principles in *Datura* was analyzed in the conditions of the plant's relationship with influencing factors, soil, water, nutrients, stress factors etc. (Ali, 1991; Moreno-Pedraza et al., 2019).

Based on the content of bioactive compounds, *Datura stramonium* has been studied as a potential allelopathic effect, in relation to different plant species from spontaneous flora and agricultural crops (Pacanoski et al., 2014).

At the same time *Datura stramonium* L. occurs in different crops, and is approached as a weed (Saayman-duToit, 2000).

The ecological plasticity and the relation of *Datura* plants with ecophysiological factors,

with other natural plant species, with nutrients and composition in minerals were studied (Benvenuti and Macchia, 1997; Bhattacharjee et al., 2004; Castillo et al., 2014; Camargi et al., 2017). Studies have been conducted on competition relations between *Datura* plants and different cultivated plant species (Cavero et al., 2002; Karimmojeni et al., 2010).

The effect of different herbicides on *Datura* plants was also analyzed, in order to control weeds in agricultural crops (Sakadzo et al., 2018).

The present study analyzed the species *Datura stramonium* L. to describe models of leaf surface determination and to describe the fractal geometry of the leaves.

MATERIALS AND METHODS

Biological material was represented by the species *Datura stramonium* L. Leaf samples were taken from various mature plants, from the agricultural area adjacent to Timisoara, Timis County.

To obtain the values of dimensional parameters, such as length (L) and width (w), the leaves were measured with an accuracy of ± 0.5 mm.

The leaves were scanned in a 1:1 size ratio. Leaf images were analyzed to obtain data regarding perimeter (Per) and scanned leaf area (SLA).

The fractal geometry of the leaves was analyzed on binarized images, Figure 1, by the box-counting method (Voss, 1985).



Figure 1. *Datura stramonium* L. leaf sample (binarized image representation)

Experimental data were analyzed to assess the presence of variance and data safety. Also, correlations between determined foliar parameters, interdependence relations, and prediction relations were analyzed. ANOVA test, correlation analysis, regression analysis were used. Correlation and regression coefficients (r , R^2), parameter p and RMSEP were used as parameters for estimating the statistical safety of the results.

ImageJ (Rasband, 1997), PAST (Hammer et al., 2001), and Wolfram Alpha (2020) softwares were used for image analysis and statistical data processing.

RESULTS AND DISCUSSIONS

From the determinations made at the leaf samples, *Datura stramonium* L. species, different values were registered for the leaf length $L = 6.90-12.40 \pm 0.147$ cm, and leaf width $w = 4.90-9.70 \pm 0.109$ cm, for the leaf perimeter $Per = 23.273-51.978 \pm 0.640$ cm, for the scanned leaf area $SLA = 17.21-67.38 \pm$

1.088 cm^2 and for the measured leaf area $MLA = 17.25 - 60.14 \pm 0.998 \text{ cm}^2$ respectively. The graphic distribution is presented in Figure 2.

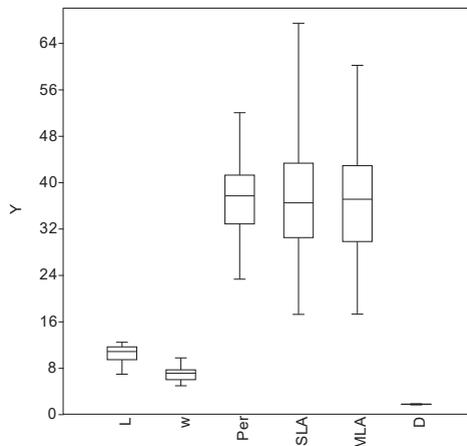


Figure 2. Graphical representation as box-plot type, of the values of the studied foliar parameters of the species *Datura stramonium* L.

The statistical safety of the experimental data and the presence of variance in the data set were evaluated by the ANOVA Test ($F > F_{crit}$; $p < 0.001$), for $\text{Alpha} = 0.001$. The experimental data, in the case of the studied leaf parameters, had a normal distribution.

A non-destructive method, very accessible for determining the leaf area is based on the parameters of the leaves (length - L , width - w). The relationship between L and w leads, in the case of leaves, most frequently to a rectangular geometric surface. The leaf falls within the respective geometric surface, but the relationship requires a correction to render only the actual surface of the leaf. In this sense, a specific correction factor (CF) is required. In order to find out the optimal value of the CF, the model proposed by the Sala et al. (2015) was used. The correction factor has subunit values ($CV < 1$). In the present study, a narrow range (0.45, 0.55) was identified by preliminary calculations. During this interval, calculations were made with a variation of 0.1 units of CF to find the MLA (measured leaf area), relation (1).

$$MLA = L \cdot w \cdot CF \quad (1)$$

The MLA values obtained by calculation were compared with the SLA values, and at $CF = 0.50$ the minimal errors means (MEM) was

registered. This confirmed that the value 0.50 represented the optimal value for CF, Table 1, Figure 3.

Table 1. MLA values in relation to CF for *Datura stramonium* L. leaves

CF values	SLA	MLA	MEM	RMSEP
0.45	36.221	32.887	-3.334	4.55600
0.46		33.618	-2.603	3.98023
0.47		34.349	-1.872	3.47484
0.48		35.080	-1.141	3.07473
0.49		35.811	-0.410	2.82497
0.5		36.542	0.321	2.76661
0.51		37.272	1.051	2.91116
0.52		38.003	1.782	3.23153
0.53		38.734	2.513	3.68209
0.54		39.465	3.244	4.22136
0.55		40.196	3.975	4.81966

The RMSEP statistical parameter, relation (3), also confirmed that the value CF = 0.50 represented the optimal value for the correction factor (RMSEP = 2.76661, for CF = 0.50). The RMSEP values for the series of tested CF values, are presented in Table 1.

$$RMSEP = \sqrt{\frac{1}{n} \sum_{j=1}^n (y_j - \hat{y}_j)^2} \quad (2)$$

Corresponding to CF = 0.50 value found, and of the measured leaf parameters (L and w), was calculated the value of the MLA (measured leaf area), Table 2. For CF = 0.50 was found MLA = 36.542 cm², with MEM = 0.321.

The leaves of the species *Datura stramonium* L. can be described as simple leaves, elliptical in shape, with toothed edges, with alternating arrangement on the plant. Fractal analysis, the box-counting method, was used to describe the geometry of the leaves (Voss, 1985).

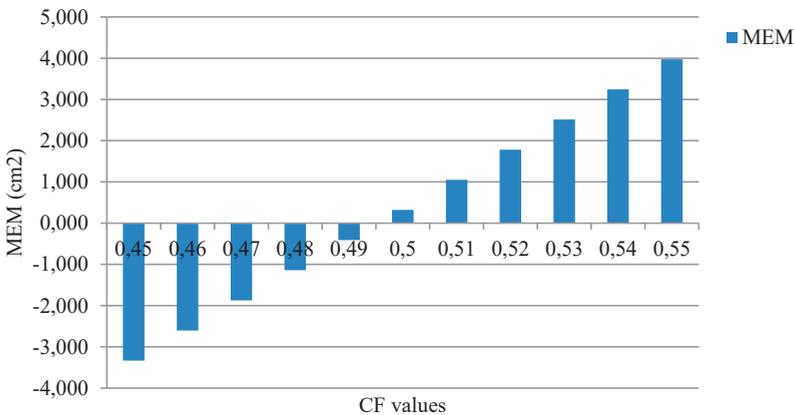


Figure 3. Graphic distribution of MEM, in the calculation of MLA based on CF values, leaf samples *Datura stramonium* L.

The binarized images of the leaf samples were analyzed, in conditions of high statistical safety, R² for D = 0.997, SE = 0.004, Table 1. The average value of the fractal dimension D that described the geometry of the leaves at *Datura stramonium* L. was D = 1.707.

Very strong and strong correlations were recorded between foliar parameters and SLA, MLA and D respectively (eg. r = 0.930 between MLA and L; r = 0.969 between MLA and w; r = 0.946 between Per and SLA; r = 0.933 between D and SLA).

The regression analysis highlighted the close relationship between the leaf parameters L, w

and SLA in the variation of the fractal geometry of the leaves, respectively in the definition of the fractal dimension (D).

The fractal dimension D, depending on the foliar parameters L and SLA, was described by equation (3), in conditions of statistical safety of the equation, according to R² = 0.999, p < 0.001. According to the ANOVA test and the values of the coefficients of equation (3) presented statistical certainty (p << 0.001 for a; p = 0.0408 for b; p << 0.001 for c; p < 0.001 for d; p = 0.0011 for e). The 3D graphic distribution is presented in figure 4, and the

distribution in the form of isoquants is shown in figure 5.

$$D_{(L,SLA)} = ax^2 + by^2 + cx + dy + exy + f \quad (3)$$

where: $D_{(L,SLA)}$ - fractal dimension, in relation to L and SLA

$x - L; y - SLA;$

a, b, c, d, e, f – coefficients of the equation (3);

a= -0.0298384699529701;

b= -0.000190689869023275;

c= 0.445520607743716;

d= -0.0353362346248073;

e= 0.00490581157233495;

f= 0.

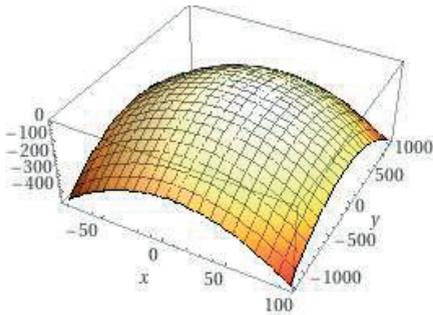


Figure 4. 3D graphical representation of the distribution of the fractal dimension D according to L and SLA, *Datura stramonium* L.

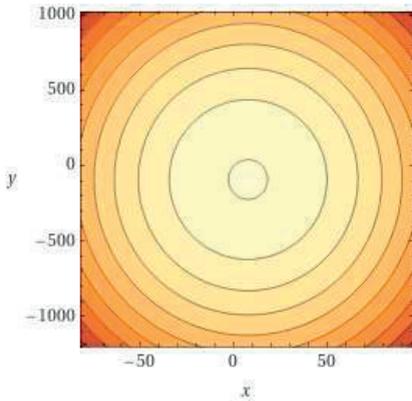


Figure 5. Graphic representation as isoquant of the distribution of the fractal dimension D according to L and SLA, *Datura stramonium* L.

The variation of the fractal dimension D depending on the foliar parameters w and SLA, was described by equation (4), in conditions of general statistical safety of the equation, according to $R^2 = 0.999, p < 0.001$. According to the ANOVA test and the values of the coefficients of equation (4) presented statistical

safety ($p \ll 0.001$ for a; $p = 0.00379$ for b; $p \ll 0.001$ for c; $p < 0.001$ for d; $p < 0.001$ for e). The 3D graphic distribution is presented in figure 6, and the distribution in the form of isoquants is presented in Figure 7.

$$D_{(w,SLA)} = ax^2 + by^2 + cx + dy + exy + f \quad (4)$$

where: $D_{(w,SLA)}$ - fractal dimension in relation to w and SLA

$x - w; y - SLA;$

a, b, c, d, e, f – coefficients of the equation (4);

a= -0.0993837006585064;

b= -0.000777662962919886;

c= 0.768644952854588;

d= -0.0549306446216847;

e= 0.016679744981095;

f= 0.

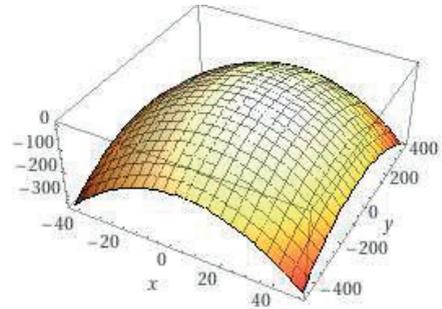


Figure 6. 3D graphical representation of the distribution of the fractal dimension D according to w and SLA, *Datura stramonium* L.

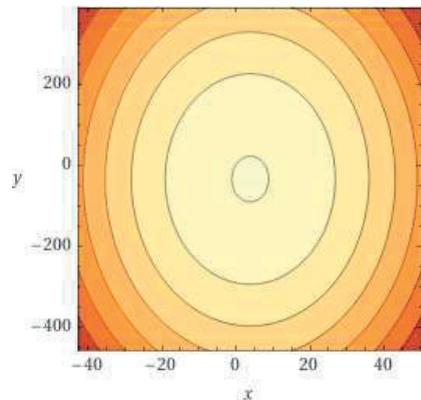


Figure 7. Graphic representation as isoquant of the distribution of the fractal dimension D according to w and SLA, *Datura stramonium* L.

The variation of the fractal dimension D in relation to the foliar parameters Per and SLA, was described by equation (5), in conditions of general statistical safety of the equation,

according to $R^2 = 0.999$, $p < 0.001$. According to the ANOVA test and the values of the coefficients of equation (5) presented statistical certainty ($p \ll 0.001$ for a; $p < 0.001$ for b; $p \ll 0.001$ for c; $p \ll 0.001$ for d; $p < 0.001$ for e). The 3D graphic distribution is presented in figure 8, and the distribution in the form of isoquants is presented in Figure 9.

$$D_{(Per,SLA)} = ax^2 + by^2 + cx + dy + exy + f \quad (5)$$

where: $D_{(Per,SLA)}$ - fractal dimension in relation to Per and SLA

x - Per; y - SLA;

a, b, c, d, e, f - coefficients of the equation (5);

a = -0.00477548971090102;

b = -0.00133694153031225;

c = 0.170424058654503;

d = -0.0793952105658202;

e = 0.00492200264906732;

f = 0.

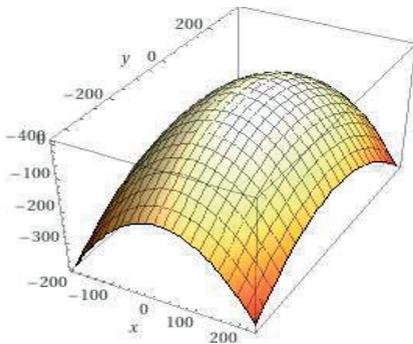


Figure 8. 3D graphical representation of the distribution of the fractal dimension D according to Per and SLA, *Datura stramonium* L.

Analyzing the foliar parameters studied in terms of coefficient of variation (CV), high values were found for SLA ($CV_{SLA} = 30.0438$) and MLA ($CV_{MLA} = 27.3120$), moderate values in the case of L ($CV_L = 14.2455$), w ($CV_w = 15.6528$) and Per ($CV_{Per} = 17.5079$). Low value of the coefficient of variation were recorded in the case of fractal dimension D ($CV_D = 2.4234$). Variations in plant leaves are generated by complex causes, such as the relationship of plants with natural and anthropogenic environmental factors (Datcu et al., 2017; Li et al., 2018; Liu et al., 2019), by foliar treatments, cultivation and growth conditions (Rawashdeh and Sala, 2014; del Pazo et al., 2020; Ren et al., 2020), by stress factors (Poorter et al., 2009) etc.

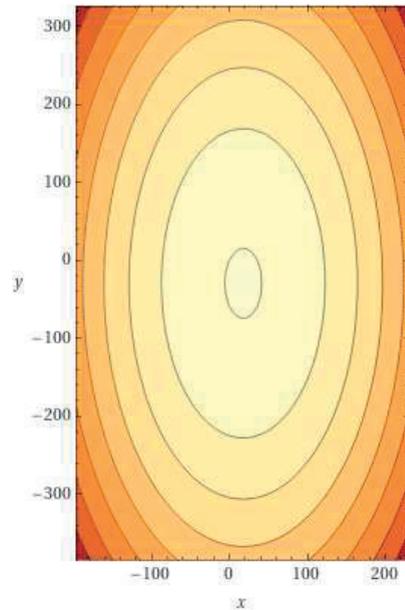


Figure 9. Graphic representation as isoquant of the distribution of the fractal dimension D according to Per and SLA, *Datura stramonium* L.

Imaging analysis is a very useful method for the study of plants at the foliar level, the whole plant, or plant associations (natural or anthropogenic ecosystems) due to the various approaches and facilities it offers; obtaining results in real time (or very short compared to classical methods), has applicability on mobile devices, high portability etc. It also allows integration into complex analysis schemes, and association with various image capture devices (Li et al., 2014; Komis et al., 2018; Beć et al., 2020).

Imaging analysis has been used for studies of leaf structure and morphology (Mathers et al., 2018), plant phenotyping (Li et al., 2014; Rousseau et al., 2015), analysis of physiological indices (Drienovsky et al., 2017a; Constantinescu et al., 2018), detection of symptoms at the foliar level and plant diseases (Drienovsky et al., 2017b; Veys et al., 2019; Singh et al., 2020), characterization of biomass in agricultural crops (Pandey et al., 2017) etc.

In the present study, based on the CV values resulting from the analysis of the leaf samples for the species *Datura stramonium* L., it can be appreciated that for the description of the leaf geometry the greatest stability was presented by the fractal dimension (D). Similar results

were reported by Sala et al. (2020a) for the black alder.

Similar studies have been conducted for the analysis of leaf fractal geometry and characterization of some plant species (Backes et al., 2009; Du et al., 2013; Gazda, 2013; Da Silva et al., 2015), and for the analysis and classification of five varieties of apple, based on the fractal geometry of the leaves (Sala et al., 2017).

Fractal analysis was used to describe the geometry of the ash tree rhytidome, and based on the fractal dimensions, a variation model was performed in relation to the shooting distance (Nicolin et al., 2019).

The geometry of some wheat plant associations, at the level of wheat crops, was studied by fractal analysis, in order to classify 10 wheat varieties (Rujescu et al., 2020). The method used facilitated the analysis and classification of the varieties studied, in conditions of statistical safety.

In the analysis of the degree of spatial variability of agricultural lands, fractal analysis led to the development of variation models based on the values of fractal dimensions D resulting from imaging analysis (Sala et al., 2020b).

The results obtained in the present study fall within the general and specific context of the studied bibliographic sources for the scientific substantiation of this research and bring information on the particular geometry of the leaves to the analysed specie, *Datura stramonium* L.

CONCLUSIONS

To find the leaf area at *Datura stramonium* L. by non-destructive methods, based on the leaf parameters L and w (measured leaf area - MLA) the value of the correction factor was found, $CF = 0.50$. This facilitated the finding of MLA values with high accuracy and in statistical safety conditions.

The description of the leaf geometry was possible by fractal analysis ($D = 1.707$), of the leaf samples taken in the study.

Very strong correlations were identified between foliar parameters of the leaves and elements of the geometry of the studied leaves (SLA, MLA, D). Mathematical models were

found that described the interdependence relationships between stidiati foliar parameters and fractal dimension D .

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