NON-DESTRUCTIVE METHOD TO DETERMINING THE LEAF AREA IN HEMP, *Cannabis sativa* L.

**Ciprian BUZNA**¹, **Florin SALA**²

¹Agricultural Research and Development Station Lovrin, Lovrin, 307250, Romania  
²University of Life Sciences "King Michael I" from Timisoara, Timisoara, Calea Aradului, 300645, Romania

*Corresponding author: florin_sala@usab-tm.ro*

**Abstract.** The evaluation of the leaf surface of hemp leaves, *Cannabis sativa* L., was the subject of the present study. The biological material was represented by the hemp variety 'Silvana', cultivated in SCDA Lovrin, Romania. Hemp leaves were taken randomly from mature plants. The leaves were herborized immediately after harvesting, in the field, in order to preserve the configuration of the leaves for analysis. Each leaf was scanned in a 1:1 ratio. The leaves were measured in terms of length (in the direction of the central leaflet) and width (maximum opening of the leaflets). For the measurement, a precision of ±0.5 mm was ensured. The scanned leaves were analyzed and the perimeter (Per) and scanned leaf area (SLA) were obtained. The correction factor (CF) was determined in order to be used for calculating the measured leaf area (MLA) by non-destructive methods. The value CF=0.27 was found, where the error as the difference between MLA and SLA (considered as a reference) was minimal (ME=0.87 cm²). The calculation of the RMSEP parameter confirmed the value of the correction factor (0.27), at which the RMSEP value was the minimum (RMSEP=9.74929). The fitting relationship between MLA and SLA was described by a linear equation, under conditions of R²=0.952, p<0.001. Polynomial equations of the 3rd degree described the interdependence relationship between SLA and parameters L (R²=0.932) and w (R²=0.964) of the leaves, and a polynomial equation of the 2nd degree described the relationship between SLA and Per (R²=0.995). Stronger correlation levels were identified between MLA and dimensional parameters of leaves (L, w; r=0.969 for MLA with L; r=0.974 for MLA with w), than between SLA and dimensional parameters of hemp leaves (r=0.927 for SLA with L; r=0.957 for SLA with w). This difference can be explained based on the methods of determining SLA (by scanning) and MLA (by measurement, based on L, w, CF), MLA having a direct relationship between L and w from the calculation relationship, a fact that confirms the usefulness of the work method.

**Keywords:** compound leaf, hemp leaves, leaf area, leaf parameter, model, non-destructive method

**INTRODUCTION**

The concerns for the study of the leaf surface in plants are numerous, for different reasons to characterize the plants, or to analyze and evaluate production processes in relation to influencing factors (Lone et al., 2011; Liu et al., 2022).

For the study of plant leaves, under the aspect of geometry, of the leaf surface, different methods, models and formulas were used to facilitate accessible determinations, with high precision, in relation to the purpose of the research (Halilou et al., 2016; Shi et al., 2018; Yu et al., 2020).

Leaf surface study methods, based on artificial intelligence (AI), some with mobile applications, have been used to evaluate the leaf limb, or associated indices, in relation to the determination of leaf surface, plant health or crop productivity (Drienovsky 2017a,b; Ercanli et al., 2018; Castro-Valdecantos et al., 2022; Sabouri and Sajadi, 2022).

Some models were adapted to evaluate with high precision (R²=0.994) the total leaf surface of the leaves on the shoot, a situation in which the leaves have variable sizes in relation to the length of the shoot and the position on the shoot (Koyama and Smith, 2022).

In the case of cultivated species, the leaf surface is frequently analyzed and studied in relation to the characterization of certain cultivations, the state of plant nutrition, physiological processes, the productivity of plant production processes (Dobrei et al., 2016; Teobaldelli et al., 2020).

Different specific indices associated with the leaf surface, such as SLA, TDM, DBAR, were used to characterize certain plant species as bioindicators in relation to urban ecosystems (Datcu et al., 2017; Zhu and Xu, 2021).

Leaf morphology shows a high variability, and compound leaves represent special types and patterns that have been studied in relation to genetic determination and hormonal actions in model species (Efroni et al., 2010; Wang and Chen, 2014). The authors considered the purpose of the studies to facilitate the understanding of some regulatory mechanisms and to open perspectives for the study and approach of these types of leaves.

Hemp leaves, *Cannabis sativa* L., are compound leaves, and have been studied in relation to the position on the plant, age, plant nutrition, concentration of some nutrients (eg N), chlorophyll, influence on photosynthetic processes etc. (Tang et al., 2017, 2018; Bauerle et al., 2020).
The present study evaluated the leaf area of hemp composite leaves by scanning and based on leaf parameters, and determined the correction factor (CF) useful for calculating the leaf area by non-destructive methods (measured leaf area – MLA) in studies of plant dynamics, directly in field conditions.

MATERIAL AND METHODS
The study approached a non-destructive method for determining the folial surface of hemp, *Cannabis sativa* L. The biological material was represented by the Silvana variety. The culture was carried out within SCDA Lovrin, Timis County, Romania, on a medium-fertility chernozem type soil, under the conditions of the 2021 – 2022 agricultural year.

Representative leaf samples (10 samples) were taken randomly from mature plants. The leaves were herborized immediately after sampling in the field, to ensure the integrity of the leaves for further analysis, figure 1.

![Hemp Leaf Sample](image)

**Figure 1.** The hemp leaf sample, *Cannabis sativa* L., used in the study to determine the leaf surface

Each leaf was scanned in a 1:1 ratio, and images were obtained at a resolution of 300 dpi, jpeg format. The images of the leaves were analyzed to obtain the scanned leaf surface - SLA (Rasband, 1997). In addition, the perimeter of the leaves (Per) was determined.

For each leaf, measurements were made regarding the length of the central leaflet (considered leaf length – L) and the width of the leaf (w) at the tip of the leaflets that gave the maximum opening.

In order to find out the leaf surface by the non-destructive method, it was considered necessary to determine the value of the correction factor (CF) specific to hemp leaves, Silvana variety, useful in determining the measured leaf surface (MLA) based on the general relationship (1).

\[
\text{MLA} = \text{L} \times \text{w} \times \text{CF}
\]

In order to find the optimal value for CF, the model proposed by Sala et al. (2015) was considered. The obtained data were processed and analyzed mathematically and statistically to find out the optimal value for CF. Comparative analyzes were also made between SLA and MLA to find out the mean error (ME). To confirm the optimal CF value, RMSEP values were calculated for a series of 10 pairs of values.

The ANOVA test was used, as well as other appropriate statistical tests and parameters to confirm the results. The PAST software (Hammer et al., 2001) and the calculation module in EXCEL were used for data processing and the generation of graphs.

RESULTS AND DISCUSSIONS
From the measurement of the hemp leaves and the analysis of the scanned images, the values for the leaf length (L), leaf width (w), perimeter (Per), scanned and measured leaf surface (SLA, MLA) were obtained, with the graphic representation in figure 2. The basis of calculations was determined the optimal value for the correction factor (CF) and for the measured leaf area (MLA), table 1, figure 3.

For the safety of the MLA values, in relation to the SLA and the calculation precision, the mean error
values (ME) were calculated, as the difference between the MLA and the SLA, at different CF values. Also, for statistical safety, values were calculated for the RMSEP parameter, table 1.

![Graphs showing MLA and SLA values](image)

**Figure 2.** The graphic distribution, in box plot format (a), and in normal probability plot format (b), for foliar parameters in the hemp leaf samples studied, *Cannabis sativa* L., Silvana variety

<table>
<thead>
<tr>
<th>SLA (cm²)</th>
<th>CF</th>
<th>MLA (cm²)</th>
<th>ME (cm²)</th>
<th>RMSEP</th>
</tr>
</thead>
<tbody>
<tr>
<td>73.92</td>
<td>0.22</td>
<td>60.94</td>
<td>-12.98</td>
<td>18.77257</td>
</tr>
<tr>
<td>73.92</td>
<td>0.23</td>
<td>63.71</td>
<td>-10.21</td>
<td>16.30406</td>
</tr>
<tr>
<td>73.92</td>
<td>0.24</td>
<td>66.48</td>
<td>-7.44</td>
<td>14.02828</td>
</tr>
<tr>
<td>73.92</td>
<td>0.25</td>
<td>69.25</td>
<td>-4.67</td>
<td>12.0549</td>
</tr>
<tr>
<td>73.92</td>
<td>0.26</td>
<td>72.02</td>
<td>-1.90</td>
<td>10.5549</td>
</tr>
<tr>
<td>73.92</td>
<td>0.27</td>
<td>74.79</td>
<td>0.87</td>
<td>9.749295</td>
</tr>
<tr>
<td>73.92</td>
<td>0.28</td>
<td>77.56</td>
<td>3.64</td>
<td>9.810654</td>
</tr>
<tr>
<td>73.92</td>
<td>0.29</td>
<td>80.33</td>
<td>6.41</td>
<td>10.72411</td>
</tr>
<tr>
<td>73.92</td>
<td>0.3</td>
<td>83.10</td>
<td>9.18</td>
<td>12.30128</td>
</tr>
<tr>
<td>73.92</td>
<td>0.31</td>
<td>85.87</td>
<td>11.95</td>
<td>14.3246</td>
</tr>
<tr>
<td>73.92</td>
<td>0.32</td>
<td>88.64</td>
<td>14.72</td>
<td>16.63202</td>
</tr>
</tbody>
</table>

A matching relationship was found between MLA and SLA, described by equation (2), under conditions of $R^2=0.952$, $p<0.001$, $F=158.4$. The graphic representation of the MLA values in relation to the SLA and the fitting line are given in figure 4.

$$\text{MLA} = 0.788 \cdot \text{SLA} + 16.543 \quad (2)$$

The correlation analysis led to the values presented in table 2. Very strong and strong correlations were found between the foliar parameters considered in the study. The highest value for the correlation coefficient was recorded in the case of SLA with Per ($r=0.996$).
Figure 3. The graphic representation for the mean error (ME), in relation to the correction factor (CF), in hemp leaves, Cannabis sativa L., Silvana variety

Figure 4. Graphical distribution of MLA values in relation to SLA and of the straight line, hemp leaves, Cannabis sativa L., Silvana variety

Table 2. Correlation values between foliar parameters in hemp, Cannabis sativa L., Silvana variety

<table>
<thead>
<tr>
<th></th>
<th>L</th>
<th>w</th>
<th>Per</th>
<th>SLA</th>
<th>MLA</th>
</tr>
</thead>
<tbody>
<tr>
<td>L</td>
<td>0.000352</td>
<td>0.000445</td>
<td>0.000116</td>
<td>3.74E-06</td>
<td></td>
</tr>
<tr>
<td>w</td>
<td>0.902</td>
<td>2.53E-05</td>
<td>1.45E-05</td>
<td>1.87E-06</td>
<td></td>
</tr>
<tr>
<td>Per</td>
<td>0.896</td>
<td>0.950</td>
<td>9.53E-10</td>
<td>8.66E-06</td>
<td></td>
</tr>
<tr>
<td>SLA</td>
<td>0.927</td>
<td>0.957</td>
<td>0.996</td>
<td>1.49E-06</td>
<td></td>
</tr>
<tr>
<td>MLA</td>
<td>0.969</td>
<td>0.974</td>
<td>0.962</td>
<td>0.976</td>
<td></td>
</tr>
</tbody>
</table>

The variation of SLA was analyzed in relation to determined leaf parameters (L, w and Per). The
variation of SLA in relation to L was described by equation (3), under conditions of $R^2=0.932$, $p<0.001$, $F=27.527$. The variation of SLA in relation to w was described by equation (4), under conditions of $R^2=0.964$, $p<0.001$, $F=54.034$, and the variation of SLA in relation to Per was described by equation (5), under conditions of $R^2=0.995$, $p<0.001$, $F=760.51$.

The graphic representation of the SLA variation is presented in figure 5 (a) in relation to L, in figure 5 (b) in relation to w and in figure 5 (c) in relation to Per. From the analysis of $R^2$ and F-test values, related to equations (3) - (5), it was found that the variation of SLA in relation to Per presented the highest level of statistical safety.

\[
\text{SLA} = -0.215x^3 + 11.06x^2 - 172.2x + 884.4 \quad (3)
\]

\[
\text{SLA} = -0.1001x^3 + 5.878x^2 - 102.5x + 594.5 \quad (4)
\]

\[
\text{SLA} = -0.0005752x^2 + 0.6901x - 38.4 \quad (5)
\]

where:
- $x$ – leaf length (L) in equation (2);
- $x$ – leaf width (w) in equation (3);
- $x$ – leaf perimeter (Per) in equation (4).

Figure 5. Graphic distribution of SLA in relation to foliar parameters, L (a), w (b), and Per (c) in hemp, *Cannabis sativa* L. Silvana variety
The distribution of MLA in relation to L and w, obtained by graphic analysis, is presented in figure 6, and from the analysis of the distribution of the samples taken in the study, it can be observed the increase of the leaf surface with a certain proportionality with leaf parameters L and w.

Figure 6. Graphical distribution of MLA values in relation to L and w, hemp leaf samples, *Cannabis sativa* L., Silvana variety

The determination of the leaf surface based on the parameters of the leaves (L, w) and a correction factor has been used in numerous studies, as a result of the accessible method for the study of the leaf surface and the high precision.

Sala et al. (2015) communicated a model for finding the values of the surface constants (K_A) for five apple varieties, and based on the calculated K_A values it was possible to accurately determine the values of the measured leaf area (MLA). The authors communicated different levels of correlation between the studied leaf parameters and the leaf surfaces determined by scanning (SLA) or by measurement (MLA), as well as a higher level of confidence in the prediction of leaf area based on leaf parameters L and W. Based on the values of the coefficient of determination (R^2), a higher level of confidence was found in the leaf area prediction based on the W parameter (R^2=0.995) compared to the leaf area prediction based on the L parameter (R^2=0.987). By studying the geometry of leaves through fractal analysis, Sala et al. (2017) obtained the classification of the analyzed apple varieties under statistical safety conditions (Coph. corr.=0.967).

Carvalho et al. (2017) communicated linear models for predicting leaf area based on leaf parameters in *Crotalaria juncea*, under statistical safety conditions (R^2=0.984).

In four energetic poplar clones, distinct values were obtained for the surface constant (k), between k=0.62 and k=0.74, values that facilitated the high-precision calculation of the measured leaf surface - MLA (Cândea-Crăciun et al., 2018). The authors communicated high levels of statistical confidence between leaf parameters, as well as leaf surface (r=0.930 for L and W; r=0.960 for SLA with Per).

Different relationships of proportionality between the leaf surface and leaf parameters have been communicated in relation to different typologies of the leaf lamina (Shi et al., 2019).

Teobaldelli et al., (2020) reported high levels of statistical confidence in estimating leaf area based on leaf width (W), under conditions of R^2=0.948 for basil, R^2=0.963 for mint, and R^2=0.925 for sage. Under the conditions of using the L and W parameters of the leaves (length, width), the authors communicated the best accuracy and fitting, with values of the statistical safety parameters of R^2=0.992 and RMSE=0.327 for basil; R^2=0.998 and RMSE=0.222 for mint, respectively R^2=0.998 and RMSE=0.426 for sage (RMSE value in cm^2).

The results obtained in the present study, regarding the determination of the correction factor (CF) and the calculation of the measured leaf area (MLA), based on the leaf parameters (L and w) and CF, contribute to the
database regarding the determination of the leaf area in plants by non-destructive methods, useful in conditions of field experiments, in determining the growth dynamics of plants without sacrificing them, or in the case of few specimens of plants that require further observations.

CONCLUSIONS
The samples of hemp leaves, *Cannabis sativa* L. due to the specific sensitivity after detachment from the plants, require herbalization immediately after sampling, in order to ensure physical integrity for laboratory determinations for dimensional parameters, and scanning for imaging analysis.

Based on some leaf samples, under preliminary test conditions, it was possible to determine the leaf parameters *L*, *w* by measurement, respectively *Per* and *SLA* by imaging analysis on scanned images, for the purpose of a comparative analysis under statistical safety conditions. Through comparative analysis methods, the correction factor (CF) specific to the leaf samples studied for hemp, *Cannabis sativa* L., Silvana variety, was determined, in the amount of CF=0.27.

Based on direct relationships between *L* and *w*, adjusted by CF, it was possible to calculate the measured leaf area (MLA) with high precision, verified based on the error between SLA and MLA, and the RMSEP parameter (RMSEP=9.74929).

The study method will be developed in subsequent studies, to study the typology of the compound leaves of hemp, *Cannabis sativa* L., for the purpose of applicability to different varieties approved and in cultivation, as well as for a higher level of statistical safety.

ACKNOWLEDGMENTS
The authors thank SCDA Lovrin resort for facilitating the conduct of this study.

REFERENCES