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VARIABILITY OF SOME LEAF PARAMETERS IN *LIQUIDAMBAR STYRACIFLUA* L. IN CONDITIONS OF LEAVES CHLOROSIS

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Abstract

The study analyzed the variability of some leaf parameters in the species Liquidambar styraciflua L. under conditions of leaf chlorosis. Leaf samples (normal and chlorotic) were taken from trees in the urban area, Timisoara Municipality. A differentiated variation of the values of the determined leaf parameters was recorded: leaf length, L = 6.15 – 15.00±0.42 cm; scanned leaf area, SLA = 23.68 – 132.48±5.27 cm^2; photosynthetic pigments, Chl = 1.77 – 50.36±3.23, Car = 1.76 – 10.16±0.52; fresh weight, Fw = 0.327 – 3.127±0.127 g; dry weight, Dw = 0.058 – 1.120±0.052 g; specific fresh weight, SFw = 0.0138 – 0.0236±0.0005 g cm^{-2}; specific dry weight, SDw = 0.0024 – 0.0087±0.0003 g cm^{-2}; fresh weight to dry weight ratio, Fw/Dw = 2.6012 – 5.6379±0.1755; specific dry weight to specific fresh weight ratio, SDw/SFw = 0.1774 – 0.3844±0.0119. Correlation of different levels of intensity was recorded between the analyzed parameters, and the regression analysis led to models in the form of equations and graphic form, under conditions of statistical safety (p<0.001). According to PCA, distribution diagrams were generated in relation to categories of leaf parameters, in which the main components (PC1, PC2) explained the presence of variance.

Key words: *Liquidambar, leaf chlorosis; models, PCA, photosynthetic pigments, specific dry weight.*

INTRODUCTION

Leaf chlorosis represents a quality of life problem for plants, as a result of some interferences and imbalances in the synthesis of chlorophyll. Low levels of chlorophyll are the main factors of plant nutrition imbalances, abiotic stress or inadequate water quality (Abbasi et al., 2023).

Chlorophyll deficiency in leaves affects the photosynthetic process, plant growth and development, plant productivity, harvest quality. In the case of ornamental plants, their ornamental appearance is affected.

Chlorosis is associated, most frequently, with the deficient absorption of iron and the deficient supply of the plant organism this nutrient (Li et al., 2021; Yoneyama, 2021).

Chlorosis has been studied in different plants from the spontaneous flora or plants of economic interest, herbaceous or arboreal, such as grasses (barley, rice), palm species, plane trees, petunias, aromatic plants, and many other species (Šrámek and Dubský, 2009; Aalipour et al., 2019; Li et al., 2021; Yoneyama, 2021; Mehrotra et al., 2022).

The manifestation of chlorosis is varied, from a few leaves, to branches, parts of the crown of trees or even to entire trees, depending on the growing conditions associated with the soil, but also the favorable factors (Koenig and Kuhns, 2002). The authors communicated that under the conditions of maintaining chlorosis for long periods (sometimes for several years), individual elements of the crowns of the trees can dry out, and in some cases the entire tree plant can die.

In experimental conditions, with different types of substrate, fertilization and pH of the substrate, the intensity of petunia chlorosis was evaluated (Šrámek and Dubský, 2009). The authors of the study reported the presence and variation of the intensity of chlorosis in plants with the increase in the pH value associated with the addition of limestone, but also associated with some substrate components (pH = 4.4, optimal value for petunias, under the study conditions). The reduction of chlorosis was registered under the conditions of the application of nutrient solutions with different microelements (including Fe, EDTA chelated form).

The phenomenon of leaf chlorosis in some ornamental plants was studied in relation to the application of some hormonal treatments (s-ABA) (Waterland et al., 2010). The authors of the study recorded the variation of chlorosis intensity in relation to the plant species, the time and the concentrations of s-ABA applied.

The manifestation of chlorosis was studied in *Oxalis regnellii* (ornamental potted plant) in relation to viral infections, nutritional deficiencies with Fe and Mn (analyzed independently and in association), or with growth conditions, e.g. temperature (Miller et al., 2012). Through the comparative analysis of the considered factors, the authors elucidated the initial hypotheses, and communicated the favorable response of the plants to the treatments with foliar products with Fe (EDDHA).

Symptoms that expressed plant deficiencies, including chlorosis, were studied by PCR tests on different ornamental plants (Khasa et al., 2016). The prevention and correction of chlorosis was studied in the pea crop under the conditions of application of different fertilizing products, at various times of vegetation, with the formulation of effective technological recipes (Kabir et al., 2016). The phenomenon of chlorosis (severe chlorosis) was studied in tomatoes in relation to the spectrum and light intensity (Pham et al., 2019). The authors of the study recorded the variation in the level of chlorotic intensity of the leaves depending on the light conditions of the plants (blue or red - monochromatic; combined). High intensity of chlorosis was recorded under the conditions of plant growth in blue, monochromatic light.

Chlorosis was analyzed in accordance with the state of nutrition and certain physiological characteristics of the plane tree, under experimental conditions under the influence of some variants of organic, mineral and mycorrhization fertilization (Aalipour et al., 2019). The authors of the study recorded a high level of chlorosis in the control variant, and reduced values in the fertilized and mycorrhized variants.

Genetic analyzes were done to understand the genetic mechanisms that intervene in the regulation of poplar (*Populus deltoides*) leaf color, with the variation and degradation of chlorophyll content (Zhang et al., 2019). Vaid et al. (2020) used a genetic approach to study genes involved in hybrid chlorosis in *Arabidopsis thaliana*.

Morphological and physiological changes (e.g. reduction of chlorophyll content, reduction of fresh weight) associated with iron deficiency and chlorosis, was communicated by Li et al. (2021) in palm (*A. catechu*) seedlings. The authors of the study identified the sensitivity of the species to iron and communicated possible mechanisms of plant adaptation to iron deficiency, through the synthesis and accumulation of flavonoids and organic acids.

The phenomenon of chlorosis was studied in rice in relation to fixed and variable doses of N (Kharim et al., 2020). The authors reported the lowest level of chlorosis in the case of variable doses of nitrogen, compared to fixed doses.

Chlorosis, associated with Fe deficiency in alkaline soil conditions, has been studied in different species of aromatic plants (Mehrotra et al., 2022). Such crops often occupy marginal land, affected by various limitations, including soil reaction, nutrient content and the availability of mineral elements for plant nutrition. The study presented by Mehrotra et al. (2022) shows interest from the perspective of formulating technological recipes (foliar fertilizers, chelated products, nanoparticles) for the efficiency of cultivating these categories of plants. Biocompatible Fe nanoparticles have complex effects on plant metabolism, in addition to direct iron supplementation (Sala, 1999; Gracheva et al., 2023).

Associated with the spring and autumn seasons, chlorosis (different levels of intensity, from normal to severe chlorosis) was recorded and studied in citrus species (Xiong et al., 2023). The authors reported different metabolic and physiological disorders associated with chlorosis. Different methods and techniques are used to evaluate chorosis, based on visual inspection, foliar diagnosis (leaf samples and laboratory determinations), imaging analyzes (Abbasi et al., 2023).

This study analyzed the phenomenon of chlorosis in the leaves of *Liquidambar styraciflua* L. in relation to the content of photosynthetic pigments and leaf parameters and physiological indices.

MATERIALS AND METHODS

In relation to the purpose of the study, leaf samples were taken from *Liquidambar styraciflua* L. trees, which showed symptoms of chlorosis. For comparison, normal leaves were also taken. The leaf samples were taken in July 2022, from ornamental trees, public domain, Timisoara Municipality. Examples from the set of leaf samples are shown in Figure 1.

Figure 1. Examples of chlorotic and normal leaf samples, *Liquidambar styraciflua* L.

In order to characterize the leaves in relation to the chlorotic state, several determinations were made. Dimensional parameters of the leaves were determined (length – L, scanned leaf surface – SLA) (Rasband, 1997).
The photosynthetic pigments were determined, namely the chlorophyll content (Chl) and the carotenoid content (Car). The determinations were made with SPAD-502Plus devices (KONICA MINOLTA), and respectively with ACM-200 Plus (OPTI-SCIENCES).
Fresh weight (Fw, g) was determined by weighing each fresh leaf, and dry weight (Dw, g) was determined by weighing each dry leaf. Drying and weighing of the leaf samples was done with an AXIS thermal balance (ATS 60 model, Gdańsk, Poland). Parameters Fw and Dw were determined for each leaf.
Starting from the value of the leaf area (SLA) and the Fw, respectively Dw values, related to each leaf, SFw (Specific fresh weight, g cm^{-2}) and SDw (Specific Dry weight, g cm^{-2}) parameters were calculated. Based on the determined parameter values, the ratios Chl/Car, Fw/Dw and SDw/SFw were calculated.
The recorded data were analyzed and interpreted mathematically and statistically appropriately, in relation to the purpose of the study (Hammer et al., 2001; JASP, 2022).

RESULTS AND DISCUSSIONS

Leaf samples (*Liquidambar styraciflua* L.) taken from trees that showed symptoms of chlorosis and from trees with normal vegetation were analyzed to determine some biometric parameters (L, SLA), the content of photosynthetic pigments (chlorophyll - Chl, carotenoids - Car), of the weight of the fresh substance (Fw) and of the dry substance (Dw). Based on the recorded values, the ratios between chlorophyll and carotenoids (Chl/Car), between fresh substance and dry substance (Fw/Dw) and between Specific dry weight and Specific fresh weight (SDw/SFw) were calculated.
The recorded experimental data were analyzed statistically, and the resulting values are presented in Table 1. The recorded results showed statistical certainty, according to ANOVA Test, Table 2.
Regarding the chlorophyll (Chl) content, six leaf samples (leaf sample Ls1 to Ls6) were included in the 1st quartile (lower quartile), which presented the lowest chlorophyll content (Chl < 7.800 SPAD). These samples showed a very strong chlorosis.
In the 2nd quartile (same as the median zone), 12 leaf samples (Ls7 to Ls18) were included, which presented the chlorophyll content with values close to the median (Median = 23.48), (Table 1), respectively Chl = 7.800-34.998.
Six leaf samples (Ls19 to Ls24) were included in the 3rd quartile (upper quartile), which showed high values of chlorophyll content, Chl > 34.998. The graphic distribution of the chlorophyll content on the quartiles, is shown in Figure 2 (a).
In the case of the carotenoid content, six leaf samples (Ls1 to Ls6) were included in the 1st quartile, with the carotenoid content Car <

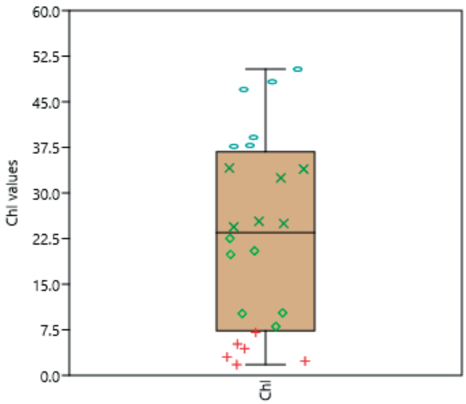
2.188. In the 2nd quartile, 12 leaf samples (Ls7 to Ls18) were included with the content of carotenoids in the median area (Median = 3.34) (Table 1), respectively Car = 2.188-4.532. Six leaf samples were included in the 3rd quartile, with carotenoid content Car > 4.532. The graphic distribution of the carotenoid content is presented in Figure 2 (b).

Table 1. The values of statistical parameters in *Liquidambar styraciflua* L. leaves

Statistical parameters	L	SLA	Chl	Car	Fw	Dw	SFw	SDw	Chl/Car	Fw/Dw	SDw/SFw
N	24	24	24	24	24	24	24	24	24	24	24
Min	6.15	23.68	1.77	1.76	0.327	0.058	0.0138	0.0024	0.98	2.6012	0.1774
Max	15.00	132.48	50.36	10.16	3.127	1.120	0.0236	0.0087	8.66	5.6379	0.3844
Sum	252.95	1700.41	550.75	96.44	29.102	7.869	0.3962	0.1010	126.40	101.5229	5.9400
Mean	10.54	70.85	22.95	4.02	1.213	0.328	0.017	0.004	5.27	4.2301	0.2475
Std. error	0.42	5.27	3.23	0.52	0.127	0.052	0.0005	0.0003	0.47	0.1755	0.0119
Variance	4.24174	665.64590	250.68030	6.53096	0.38495	0.06378	0.00001	0.00000	5.32621	0.73936	0.00339
Stand. dev	2.0595	25.8001	15.8329	2.5556	0.6204	0.2526	0.0025	0.0017	2.3079	0.8599	0.0582
Median	10.65	68.25	23.48	3.34	1.082	0.252	0.0159	0.0037	5.5300	4.1209	0.2427
25 prcntil	9.475	56.698	7.800	2.188	0.872	0.175	0.015	0.003	3.680	3.906	0.199
50 prcntil	10.65	68.245	23.480	3.340	1.082	0.252	0.016	0.004	5.530	4.121	0.243
75 prcntil	11.413	83.915	34.998	4.532	1.296	0.337	0.017	0.004	7.255	5.021	0.257
Skewness	-0.1039	0.3255	0.1856	1.6204	1.4418	1.9218	1.9314	1.6820	-0.4555	-0.4116	1.0558
Kurtosis	0.6556	0.4039	-1.2433	1.8763	2.9316	3.7081	3.3721	2.1566	-1.0109	-0.7892	0.1526
Geom. mean	10.3332	65.8508	15.7903	3.4449	1.0768	0.2602	0.0164	0.0040	4.5828	4.1388	0.2416
Coeff. var	19.5411	36.4149	68.9949	63.5979	51.1668	77.0271	14.8475	40.6953	43.8201	20.3271	23.5271

Table 2. ANOVA Test

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	256312.8	8	32039.1	2943.579	5.24E-78	3.833807
Within Groups	685.7175	63	10.88441			
Total	256998.6	71				



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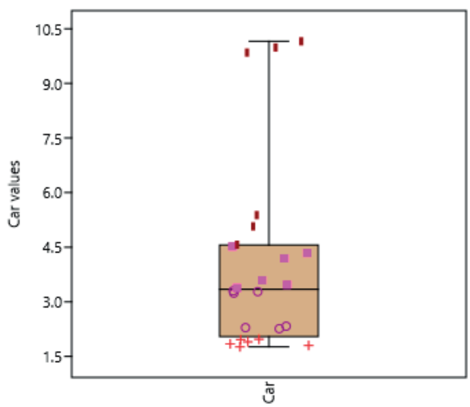

B

Figure 2. Photosynthetic pigments distributions in boxplot format, (a - Chl; b - Car), *Liquidambar styraciflua* L. leaves

Considering the determined parameters as factors in the description of the *Liquidambar styraciflua* L. leaf samples, PCA analysis was used to evaluate the importance and mode of action of the factors. Under the conditions of the data analysis, the grouping of factors resulted, according to Table 3. In the Factor 1 category, parameters were grouped (descending order of the action): SDw/SFw ratio (r = 0.893), Fw/Dw ratio (r = -0.884) , SDw (r = 0.804), Chl (r = 0.802) and Car (r = 0.761). In the Factor 2 category, parameters were grouped (in descending order of action): SAL (r = 0.900), Fw (r = 0.887), leaf length, L (r = 0.840) and dry matter, Dw (r = 0.777). The characteristics of the factors per group (Factor 1, Factor 2) are presented in Table 4. The distribution diagrams of the factors are presented in Figure 3 and figure 4.

Table 3. Factor Loadings

Parameters	Factor 1	Factor 2	Uniqueness
SDw/SFw	0.893		0.005
Fw/Dw	-0.884		0.048
SDw	0.804		0.036
Chl	0.802		0.167
Car	0.761		0.104
SLA		0.900	0.092
Fw		0.887	0.004
L		0.840	0.157
Dw		0.777	0.023
SFw			0.119
Chl/Car			0.802

Table 4. Factor Characteristics

	Unrotated solution			Rotated solution		
	SumSq. Loadings	Proportion var.	Cumulative	SumSq. Loadings	Proportion var.	Cumulative
Factor 1	8.711	0.792	0.792	4.894	0.445	0.445
Factor 2	0.738	0.067	0.859	4.549	0.414	0.858

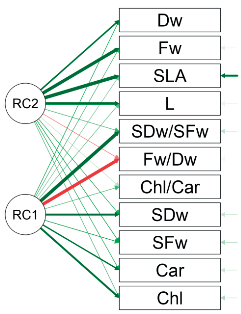

Figure 3. Path Diagram

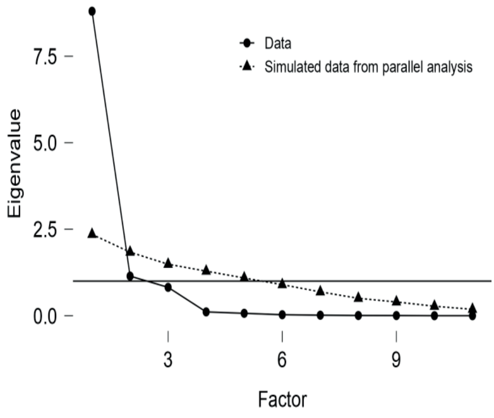

Figure 4. Scree plot

According to the value in Table 3 and the distribution in figure 2 (r = -0.884; red line), the Fw/Dw ratio has a negative effect, and the other factors have a positive effect. Specific fresh weight (SFw) and the Chl/Car ratio remained outside the two groups of factors.

Starting from the position of the parameters in relation to Factor 1 and Factor 2 (Table 3), the distribution of leaf samples (Ls1 to Ls24) was analyzed, according to the first two parameters from each group of factors.

Depending on the SDw/SFw ratio (r =0.893) and the Fw/Dw ratio (r =-0.884), the first ones ranked in Factor 1, Table 3, respectively in RC1, Figure 3, the PCA diagram in Figure 5 resulted, in which the variants were distributed in relation to the two ratios, as biplot.

PC1 explained 98.936% of variance, and PC2 explained 1.064% of variance.

According to SLA (r =0.900) and Fw (r =0.887), the first ranked in Factor 2, Table 3, respectively in RC2, Figure 3, the PCA diagram in Figure 6 resulted, in which the variants were distributed in relation with the two parameters, as biplot.

PC1 explained 97.417% of variance, and PC2 explained 2.5828% of variance.

From the analysis of the determined parameters, in the characterization of *Liquidambar styraciflua* L. leaf samples, it was found that the parameters, considered as influential factors, had a different contribution in the analysis and description of the leaf samples.

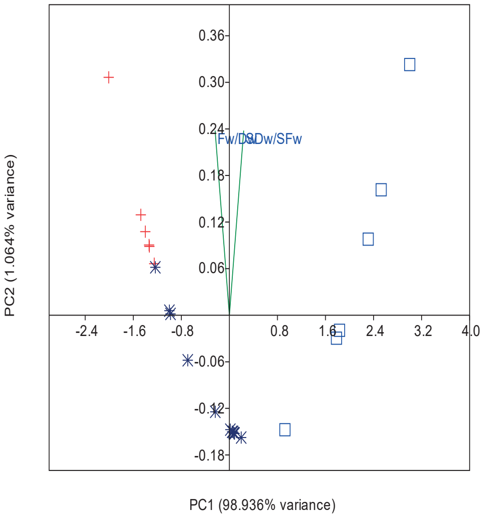

Figure 5. PCA diagram in relation to parameters SDw/SFw, and Fw/Dw, as byplot; Ls in 1st quartile - cross symbol; Ls in 2nd quartile - Star symbol; Ls in 3nd quartile - Square symbol

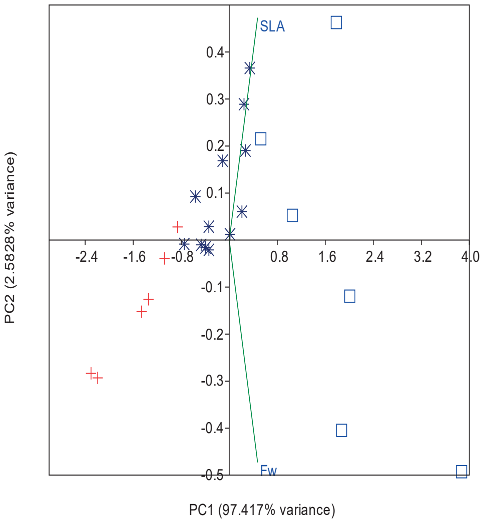

Figure 6. PCA diagram in relation to SLA parameters, and Fw, as byplot; Ls in 1st quartile - cross symbol; Ls in 2nd quartile - Star symbol; Ls in 3nd quartile - Square symbol

The length of the leaves (L) was positioned in the Factor 2 category, with positive action, of moderate intensity (r = 0.840).

The scanned leaf surface (SLA) was positioned in the Factor 2 category, with a positive, very strong action (r = 0.900). In relation to the dimensional parameters L and SLA of the leaves, it was found that the leaves with small to medium sizes, the young leaves (among the leaf samples), showed very strong and strong chlorosis.

The content of photosynthetic pigments directly expressed the level of chlorosis of the leaves. Both parameters Chl, Car) were positioned in the Factor 1 category, with positive, strong and moderate action (r = 0.802 in the case of Chl; r = 0.761 in the case of Car). The lowest values of Chl and Car were recorded in young, small leaves.

Parameters Fw and Dw were positioned in the Factor 2 category, with positive action, strong in the case of Fw (r = 0.887), and moderate in the case of Dw (r = 0.777). The Specific dry weight (SDw) parameter was positioned in the Factor 1 group, with a positive, strong action (r = 0.804).

The Specific fresh weight (SFw) parameter was not placed in the two categories of factors, similar to the Chl/Car ratio. The calculated Fw/Dw and SDw/SFw ratios were placed in the Factor 1 category, with strong positive action in the case of the SDw/SFw ratio (r = 0.893) and strong negative action in the case of the Fw/Dw ratio (r = -0.884).

Aalipour et al. (2019) reported the improvement of the photosynthetic rate by up to 60% in plane trees under the influence of fertilizer treatments and mycorrhization, compared to the control variant. Without interventions to correct chlorosis in tree species, after a period of several years of stress, Koenig and Kuhns (2002) reported the drying of some portions of the crown with negative visual/aesthetic ornamental effects, and even the drying of some trees. In the case of the present study, *Liquidambar styraciflua* L. trees were found to exhibit chlorosis in the first year, but even then corrective interventions would be recommended.

Although the primary source of chlorosis is most often the soil, foliar treatments with appropriate products generate rapid corrections (Šrámek and Dubský, 2009; Kabir et al., 2016; Mehrotra et al., 2022). In parallel with the foliar treatments, soil interventions are recommended to balance the agrochemical properties, with the aim of long-term balances for arboreal plants (ornamental trees and shrubs).

Abbasi et al. (2023) used imaging analysis to characterize chlorosis and reported an accuracy of 95% for the obtained models. The Computer

Vision models and the proposed application (based on the cloud) by the authors find practical applicability for the efficient management of crops.

CONCLUSIONS

From the analysis of the parameters determined in the leaf samples of the species *Liquidambar styraciflua* L., and their inclusion in the two categories of factors (Factor 1, and Factor 2), it was found that they presented different action in sense and intensity in relation to the chlorosis phenomenon of the leaves.

The Factor 1 category included five parameters, among which one with negative action (Fw/Dw) and four with positive action (SDw/SFw, SDw, Chl and Car). The Factor 2 category included four factors with positive action (SLA, Fw, L, Dw).

Depending on the parameters with the highest weight in Factor 1 (the ratio SDw/SFw, r=0.893, and the ratio Fw/Dw, r =-0.884), PC1 explained 98.936% of variance, and PC2 explained 1.064% of variance.

According to the first two positions in Factor 2 (SLA, r = 0.900 and Fw, r = 0.887), PC1 explained 97.417% of variance, and PC2 explained 2.5828% of variance.

The analysis of the data set showed that the smaller leaves (young leaves) showed the highest intensity of the chlorosis phenomenon, expressed by the lowest chlorophyll content of carotenoids (palsed leaf samples in the 1st quartile).

Foliar treatments associated with balanced soil interventions are necessary for the correction of chlorosis and the medium-long-term improvement of the vegetation of the studied *Liquidambar styraciflua* L. trees.

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EX·TERRA·AVRVM

MISCELLANEOUS

EX·TERRA·AVRVM