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STRUCTURAL CHARACTERISTICS AND QUANTITATIVE DETERMINATION OF THE CHLOROPHYLL IN SOME SCIOPHYTE AND HELIOPHYTE ANGIOSPERM SPECIES

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Abstract: Above all adaptive properties developed under the influence of light can be observed in the structure of the leaf blade. The structure of the above-ground stem is influenced only in an indirect way, for example it defines the level of development of the mechanical tissues. In this thesis there are histo-anatomical studies of the leaf blades and stems of 15 angiosperm species with the application of methods for making microscopically preparations. The studied vegetal material was collected from differently illuminated habitats. The determination of chlorophyll content has been made by applying the spectrophotometric method. The adaptation of the sciophytes and heliophytes leaf blades to light effects can primarily be observed in the structure, the type and in the stage of development of the assimilatory tissue. Total chlorophyll quantity was significantly higher and leaf thickness larger in sciophytes than in heliophytes.

Introduction

Beside ontogenetical stages, the internal structure of the vegetal organs is influenced in an important proportion by environmental factors, in this way during the life of a plant can appear more or less evident adaptive structural modifications depending on the conditions of the environment. Adaptations to illumination conditions become evident by the inclination to reduce the damaging effects of strong solar radiations, utilizing the light in the more useful way in case of weak illumination and by the adequate orientation in space with the aim to collect the more advantageous light energy.

The internal structural conditions in the leaf are advantageous to the function of photosynthesis. Briefly, these are, in part, the exposure of large number of chloroplasts to sunlight, the exposure of a large cell-membrane surface to the intercellular spaces where interchange of gases takes place, and such an arrangement of cells in relation to each other and to the vascular bundles that the products of photosynthesis can be rapidly removed and the cells supplied with water and mineral nutrients [3, 7, 11].

Chloroplasts are most characteristically found in the principal photosynthetic tissue, the mesophyll of leaves, but they also occur in other green parts of the plant body [1, 4, 10].

The group of chlorophyll pigments includes the chlorophylls a and b, which are found in the assimilating cells of all the plants. In the natural green leaves there is a higher quantity of chlorophyll a than b, there are almost 3 times more chlorophyll a than chlorophyll b.

Chlorophyll a, present in leaves in a larger quantity, represents the pigment able to transform light energy into chemical energy, the secondary pigments (carotenoids) yielding the absorbed energy to chlorophyll a [8].

The thylakoid disks number of the granums is high in the chloroplasts of leaves developed in low illumination, the granums are wide but they are fewer in numbers. Concerning one reaction center they have more chlorophyll molecules and in their structure are more chlorophyll-b and less xanthophyll pigment. At leaves developed in strong illumination the chlorophyll quantity referring to a surface unit is higher, but the chlorophyll quantity regarding the dry material unit is lower [5].

The quantity of the assimilatory pigments is considerably influenced by the external environment. The total assimilatory pigment quantity, their partial quantity and the ratio between them give important information about the structural integrity and physiological condition of the photosynthetic system.

By the histo-anatomical examination of the above-ground vegetative organs of plants chosen casually and developed under different illumination conditions we can study the adaptive structural characteristics formed under the influence of light and humidity, which ensure the realization of vegetal vital functions in these regions of the Ciuc-Basin.

In case of plant species occurring both in open land and in shaded places we can observe the degree to which environmental factors can influence the general organization determined by the genotype during the development of the vegetal organ.

Material and Methods

The leaves and the stems of 15 angiosperm species were examined microscopically in native cross sections and epidermal peels immediately after collection, the other sections were fixed and stained simultaneously with Congo red and chrysoidine G [6, 9].

The plants derive from Şumuleu-Mic and Şumuleu-Mare Mountains, situated in the Ciuc-Basin. The studied plants systematically belong to different families: *Lamiaceae, Scrophulariaceae, Boraginaceae, Rubiaceae, Aristolochiaceae, Ranunculaceae, Campanulaceae, Oxalidaceae, Violaceae, Fabaceae, Caryophyllaceae* and *Liliaceae.*

In the case of each species, the mature above-ground vegetative organs of 10 individuals have been histological examined. At CETI type light microscope with JVC color video camera were taken photographs of the fixed sections. The microscopical measurements were made with the help of the objective micrometer and the ocular micrometer.

Determinations of chlorophyll quantities have been executed with the spectrophotometric method, the light absorption of the samples was measured at 644 and 662 nm-s [2]. These examinations were repeated 10 times for each sample. Assimilatory pigments were extracted from mature leaves of the studied plants, capable of photosynthesis. For the extraction of chlorophyll pigments with pounding in a mortar have been used 100 mls 100 per cent aceton to 1 g of vegetal material. The studied angiosperm species are: *Asarum europaeum* L., *Oxalis acetosella* L., *Hepatica transsilvanica* Fuss., *Campanula persicifolia* L., *Viola cyanea* Čelak., *Chamaespartium sagittale* L., *Salvia pratensis* L., *Echium vulgare* L., *Galium verum* L., *Dianthus carthusianorum* L.

The light absorption values replaced in the equation below the received results show the chlorophyll concentration in mg/dm^3, which we converted into mgs of chlorophyll/g vegetal material.

$$\text{Chlorophyll a} = 9{,}78 \times A662 - 0{,}99 \times A664$$
$$\text{Chlorophyll b} = 21{,}43 \times A644 - 4{,}65 \times A662$$
$$\text{Total chlorophyll quantity} = 5{,}13 \times A662 + 20{,}44 \times A644$$

A644= light absorption measured at 644 nms.
A662= light absorption measured at 662 nms.

Two groups of species were used in the statistical analysis: sciophytes (*.Asarum europaeum, Oxalis acetosella*, *Hepatica transsilvanica, Campanula persicifolia*, *Viola cyanea*) and heliophytes (*Chamaespartium sagittale, Salvia pratensis*, *Echium vulgare*, *Galium verum*, *Dianthus carthusianorum*). Because the distribution of the data was not normal, the Kruskal-Wallis ANOVA test was used to show up differences between the two groups regarding their total chlorophyll content and leaf thickness.

Results and Discussion

The studied species live under conditions with different illumination to which they have adapted in a peculiar way considering their structure and function.

The structure of the leaves of the examined plants, collected from shaded places is dorsiventral (*Asarum europaeum, Viola cyanea*), homogenous (*Oxalis acetosella, Maianthemum bifolium, Hepatica transsilvanica*) or in *Dianthus carthusianorum* is equifacial. Epidermic cells are covered by thin cuticle (exception: *Dianthus carthusianorum*). In the case of *Oxalis acetosella, Hepatica transsilvanica* and *Maianthemum bifolium* unicellular trichomes can be observed, *Asarum europaeum* disposes of pluricellular trichomes. The anticlinal walls of epidermic cells are undulating. The mesophyll of the leaf blades of *Hepatica transsilvanica* (photo 1), *Oxalis acetosella* and *Maianthemum bifolium* are homogenous, assimilating cells under the superior epidermis contain many chloroplasts. The mesophyll of *Oxalis acetosella* and *Maianthemum bifolium* is thin (table 2), it consists of 2-5 cell layers and there are few intercellular spaces. The mesophyll of the leaf of *Hepatica transsilvanica* is thicker and in the inferior layers there are more intercellular spaces. In the leaves of *Viola cyanea* and *Asarum europaeum* the palisade parenchyma formed of one cell layer is separated from the spongy parenchyma, which fills in the rest of the mesophyll. The leaf of *Dianthus carthusianorum* collected from the forest fringe is isolateral, heterogenous and on both surfaces are developed palisade parenchyma's formed of two cell layers. In some mesophyll cells can be observed crystal inclusions too.

The mechanical tissue is missing (*Viola cyanea, Oxalis acetosella*) or it is less developed (except for *Dianthus carthusianorum* where the phloem of the vascular bundles are encircled with sclerenchyma).

The leaf structure of plants collected from open land is dorsiventral.

Photo 1: The internal structure of the leaf at *Hepatica transsilvanica* Fuss.

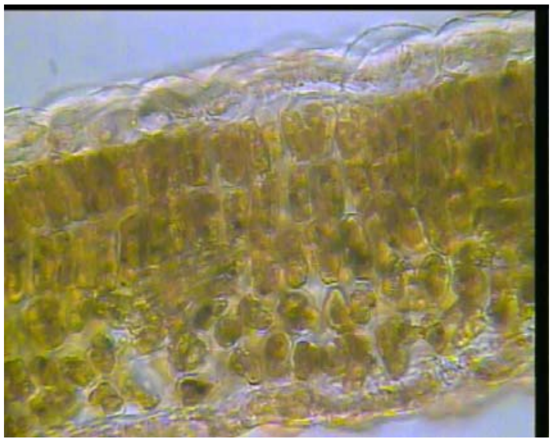

Photo 2 : Fragment of the leaf blade of *Betonica officinale* L. represented in cross section

The differentiation of the assimilatory tissues in palisade and spongy parenchyma is characteristic to the mature leaf blade, which shows a markedly heliophylic organizations (photo 2). The palisade parenchyma is well developed, being formed of one or more (example: *Betonica officinalis*), *Prunella vulgaris, Veronica spicata, Galium verum, Salvia* pratensis) cell layers. Other structural characteristics are: the anticlinal walls of the epidermic cells are not undulating, the cuticle is thick, usually trichomes can be found, and in the structure of the vascular bundles the xylem is more developed. In the case of the examined plant species mechanical tissue can be observed in the area of the principal nervure represented by the collenchyma (*Veronica spicata*) and by the sclerenchyma (*Chamaespartium sagittale*). From the leaves of *Campanula persicifolia* and *Linaria vulgaris* the mechanical tissues are missing.

The anatomical properties of the different species of leaf blades are summed up in the table 1.

Table 1: Structural characteristics of mature leaf blade of 15 angiosperm species

Species	Type of structure	The structure of the mesophyll	The type of the mechanical tissue	Type of stomatal disposure
Asarum europaeum L.	dorsiventral	heterogeneous	collenchyma	Hypostomatic
Betonica officinalis (L.) Trevis.	dorsiventral	heterogeneous	collenchyma	Amphystomatic hypostomatic (in shaded places)
Campanula persicifolia L.	dorsiventral	heterogeneous	absent	Hypostomatic
Chamaespartium sagittale L.	dorsiventral	heterogeneous	sclerenchyma	Amphystomatic
Dianthus carthusianorum L.	equifacial	heterogeneous	sclerenchyma	Amphystomatic
Echium vulgare L.	dorsiventral	heterogeneous	collenchyma	Amphystomatic
Galium verum L.	dorsiventral	heterogeneous	collenchyma	Hypostomatic
Hepatica transsilvanica Fuss.	isolateral	homogenous	collenchyma	Amphystomatic
Linaria vulgaris M.	dorsiventral	heterogeneous	absent	Hypostomatic
Maianthemum bifolium (L.) Schm.	isolateral	homogenous	sclerenchyma	Hypostomatic
Oxalis acetosella L.	isolateral	homogenous	absent	Hypostomatic
Prunella vulgaris L.	dorsiventral isolateral (in shaded places)	heterogeneous homogenous (in shaded places)	collenchyma	Amphystomatic
Salvia pratensis L.	dorsiventral	heterogeneous	collenchyma	Amphystomatic
Veronica spicata L.	dorsiventral	heterogeneous	collenchyma	Amphystomatic
Viola cyanea Čelak.	dorsiventral	heterogeneous	absent	Hypostomatic

The general organization of the leaf blades of species belonging to the same families (example: *Lamiaceae*) are identical, minimal differences can be observed in the structure of the leaf (example: the number of cell layers at the palisade parenchyma).

At plants belonging to same species (*Betonica officinalis, Campanula persicifolia, Prunella vulgaris*), occurring both in shaded places and in open lands, differences can be observed in the structure of the leaf blades first of all in the degree of development of the assimilating tissue. In the case of *Betonica officinalis*, at leaves developed under suitable illumination the palisade parenchyma was two-three cell layers, the leaf is amphystomatic, and the anticlinal walls of the epidermis cells are straight. In the leaves of plants originating from forest fringe the palisade parenchyma is only one cell layered, formed of less stretched cells (Table 3), the leaf is hypostomatic, the anticlinal walls of the epidermis cells are undulating. In the case of Prunella vulgaris, plants grown in open land have leaf blades with mesophyll separated into palisade parenchyma (two-three cell layers) and spongy parenchyma, plant living in shaded places have homogenous mesophyll. Individuals from *Campanula persicifolia* developed in open lands, in their leaf blades have one-two cell layered palisade parenchyma, the spongy parenchyma is abundant in intercellular spaces. In the leaves of individuals living under the shadow of trees and shrubs from forest fringes, near the principal nervure the mesophyll is homogenous, elsewhere it is heterogenous with one cell layer, low developed palisade parenchyma, in the spongy parenchyma there are few intercellular spaces.

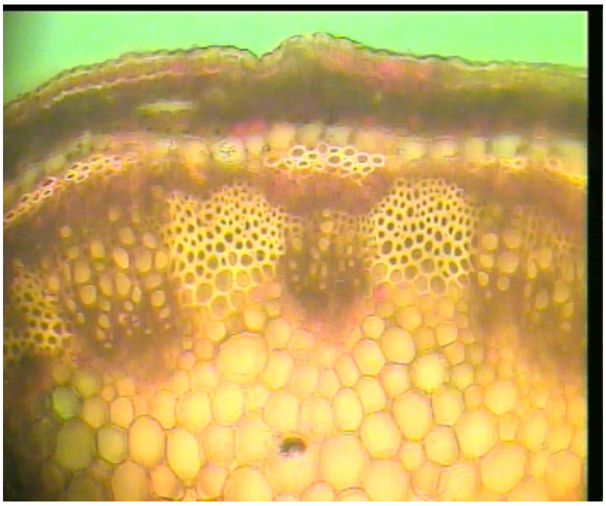

Photo 3: Internal structure of the stem at *Linaria vulgaris* M.

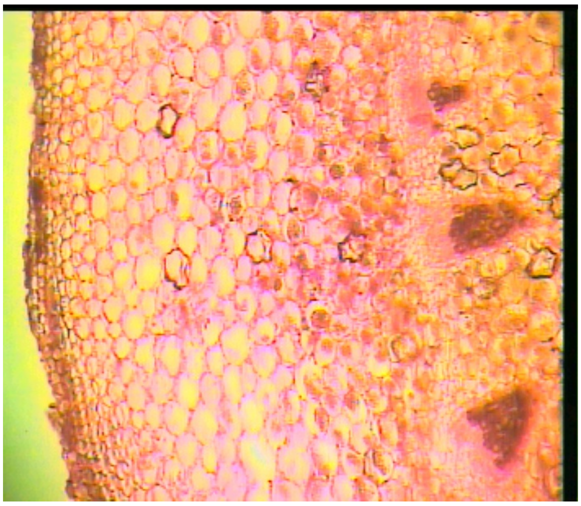

Photo 4: Cross section of stem at *Asarum europaeum* L.

The status of development of the above-ground stems chlorenchyma differs at the sciophytes and at the heliophytes alike. In the internal structure of the *Betonica officinalis*, *Dianthus carthusianorum, Campanula persicifolia, Linaria vulgaris* and *Chamaespartium sagittale* well developed chlorenchyma can be found. Types of mechanical tissues at the heliophyll species the sclerenchyma is more developed (in most of the cases it encircles the phloem of the vascular bundles) (photo 3). The mechanical tissue is missing from the *Asarum europaeum* stems (photo 4). Typical for the stem structure of the species appertaining to the *Lamiaceae* is that at the corners of the stems well developed collenchyma is formed.

Table 2: The width of leaves, mesophyll and palisade parenchyma in the studied species

Species	Leaf thickness (μm)	Mesophyll thickness (μm)	Palisade parenchyma thickness (μm)
Asarum europaeum	327,36	256,067	79,453
Chamaespartium sagittale	169,85	129,54	43,804
Dianthus carthusianorum	183,604	146,79	111,141
Echium vulgare	273,076	222,748	72,929
Galium verum	137,004	109,743	65,007
Hepatica transsilvanica	308,492	240,456	-
Linaria vulgaris	238,126	186,4	50,794
Maianthemum bifolium	142,363	99,491	-
Oxalis acetosella	102,753	49,163	-
Salvia pratensis	130,014	89,70	48,697
Veronica spicata	165,663	136,305	61,745
Viola cyanea	189,895	125,354	61,745

Our results show that in comparison with plants developed under intensive illuminated conditions the total chlorophyll quantity and the quantity of chlorophyll b is higher in those plants which grow under conditions with lower illumination. The ratio between the quantity of chlorophyll a and chlorophyll b is higher in heliophytes, because their leaves contain less chlorophyll b, this indicates the fact that under intensive light effect the peripheral pigments (where chlorophyll b is predominant) are not developed enough.

Table 4 and table 5 show the quantity of chlorophyll a, chlorophyll b and total chlorophyll in mg-s taken from 1g fresh vegetal material.

Table 3: Intraspecific variability of leaf, mesophyll and palisade parenchyma thickness

Species	**Leaf thickness** (µm)		**Mesophyll thickness** (µm)		**Palisade parenchyma thickness** (µm)	
	In shaded places	In open land	In shaded places	In open land	In shaded places	In open land
Betonica officinalis	129,781	199,681	93,433	150,052	25,863	84,113
Campanula persicifolia	206,205	224,845	149,819	158,44	40,775	65,706
Prunella vulgaris	147,256	181,041	109,743	145,858	-	65,007

Table 4: Quantities of chlorophyll extracted from sciophytes leaves

	Asarum europaeum	*Oxalis acetosella*	*Hepatica transsilvanica*	*Campanula persicifolia*	*Viola Cyanea*
Abs. (average) 644 nm	0,6619	0,515	0,4569	0,4142	0,5656
Abs. (average) 662 nm	1,5947	1,2416	1,1403	1,0455	1,3821
quantity of chlorophyll a (mg/g)	1,4949	1,1633	1,0699	0,9814	1,2956
quantity of chlorophyll b (mg/g)	0,6769	0,5262	0,4488	0,4015	0,5694
total chlorophyll quantity (mg/g)	2,1710	1,6896	1,5188	1,3830	1,8651
chlorophyll a/ chlorophyll b	2,20	2,21	2,38	2,44	2,27

Table 5: Quantities of chlorophyll extracted from heliophytes leaves

	Chamaespartium sagittale	*Salvia pratensis*	*Echium vulgare*	*Galium verum*	*Dianthus carthusianorum*
Abs. (average) 644 nm	0.5273	0,2799	0,3324	0,2895	0,6443
Abs. (average) 662 nm	1,3320	0,6662	0,8577	0,7087	1,7439
quantity of chlorophyll a (mg/g)	1,2505	0,6238	0,8059	0,6645	1,6418
quantity of chlorophyll b (mg/g)	0,5107	0,2901	0,3135	0,2909	0,56977
total chlorophylle quantity (mg/g)	1,7612	0,9140	1,1194	0,9554	2,2116
chlorophyll a/ chlorophyll b	2,44	2,14	2,57	2,28	2,88

Figure 1 show that the ten species have well definable ranges of total chlorophyll content and leaf thickness and that total chlorophyll content varies little within species, except for *Chamaespartium sagittale*. The Kruskal-Wallis ANOVA test shows that there are statistically significant differences in both the total chlorophyll content and the leaf thickness between sciophyte and heliophyte groups ($H=10.95$, $p=0.0009$ for the chlorophyll content and $H=11.68$, $p=0.0006$ for the leaf thickness). Sciophytes tend to have larger total chlorophyll content and leaf thickness than heliophytes (Fig. 2 and Fig. 3). However, *Dianthus carthusianorum* and *Chamaespartium sagittale* from the heliophilous species have high values of total chlorophyll content and *Echium vulgare* a large leaf thickness, while *Oxalis acetosella* from the sciophytes group has a low leaf thickness (Fig. 1).

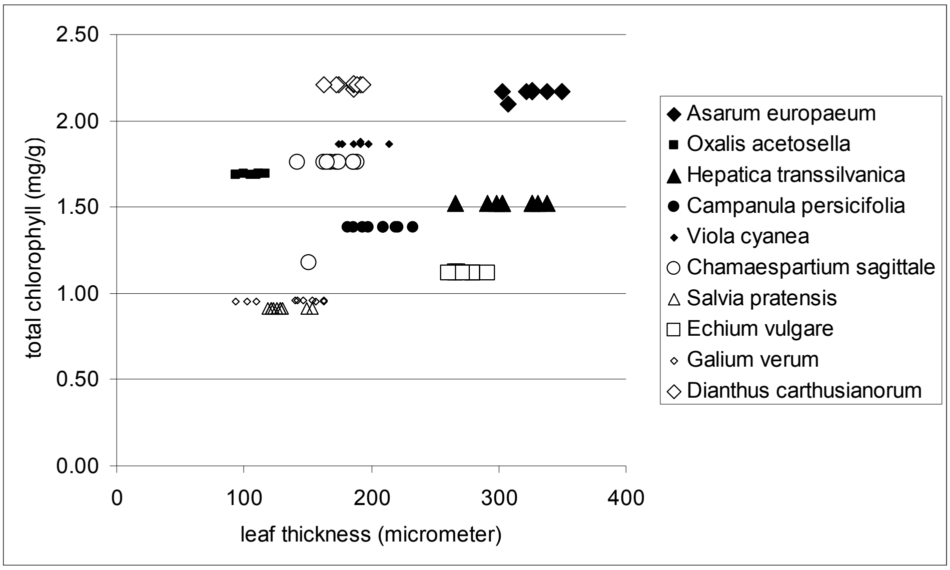

Fig. 1: Total chlorophyll content plotted against leaf thickness for sciophytes (dark symbols) and heliophytes (open symbols).

Fig. 2: Box plot of total chlorophyll content for sciophytes and heliophytes.

Fig. 3:Box plot of leaf thickness for sciophytes and heliophytes.

Conclusions

The influence of light on the internal structure of the leaf blade can primarily be observed in the structure, in the type of the assimilatory tissue and in its stage of development. The mesophyll of the leaf blades of species developed in shaded places is homogenous or the palisade parenchyma is less developed (except the equifacial *Dianthus carthusianorum* leaves, collected from forest fringe). From the examined plant species typical sciophylic characteristics are shown by the *Oxalis acetosella* and *Maianthemum bifolium* leaves. The *Betonica officinalis*, *Prunella vulgaris*, *Galium verum* and *Veronica spicata* can be included in the group of the heliophylic, where the mesophyll is mostly represented by well developed palisade parenchyma with more layers.

The structure of above-ground stems is influenced only in an indirect way.

Species belonging to the same family but exposed to different light conditions do not have essential differences in the histological structure of their above-ground organs. The palisade and the mechanical tissues are more developed in the leaf structure of the heliophytes. In the leaf structure of individuals living under different illumination conditions and belonging to the same species differences can be observed in the structure of the assimilating tissue, regarding to the position and the number of the stomata. In comparison with heliofil species, the total chlorophyll quantity and the quantity of chlorophyll-b is higher in the case of species developed in shaded places.

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STUDIUL CARACTERELOR STRUCTURALE ŞI DETERMINAREA CANTITĂŢII DE CLOROFILĂ LA CÂTEVA SPECII DE ANGIOSPERME HELIOFILE ŞI SCIAFILE

(Rezumat)

Structura limbului foliar se adaptează foarte plastic la îndeplinirea cât mai bună a funcţiei de fotosinteză şi transpiraţie, între anumite limite ale condiţiilor diferite ale mediului. Pe baza studiului anatomic pot fi făcute aprecieri asupra intensităţii unor parametri ai factorilor abiotici din mediu. Structura tulpinilor supraterane este influenţată numai în mod indirect de către lumină şi se manifestă prin prezenţa şi gradul de dezvoltare a clorenchimului şi a ţesutului mecanic. Din punct de vedere structural adaptările plantelor faţă de factorul fotic se reflectă în primul rând în structura, tipurile şi gradul de dezvoltare a ţesutului asimilator. Dintre cele 15 specii de plante studiate, cele care se dezvoltă în lumină mai puternică prezintă parenchim asimilator palisadic mai dezvoltat (*Betonica officinalis*, *Galium verum, Prunella vulgaris, Veronica spicata*), alcătuit uneori chiar din trei straturi de celule alungite, aşezate strâns unul lângă celălalt. La frunzele speciilor de plante care cresc în condiţii de iluminare slabă, parenchimul palisadic este redus (*Asarum europaeum)* sau chiar lipseşte (*Oxalis acetosella, Maianthemum bifolium*). Din punct de vedere al organizării structurale a limbului foliar în general la plantele heliofile sunt caracteristice următoarele particularităţi: cuticula este mai groasă, stomatele sunt dispuse mai rar, nervurile sunt dezvoltate, pereţii celulelor epidermice sunt drepţi, numărul vaselor lemnoase şi liberiene este mare, ţesuturile mecanice sunt dezvoltate.

Pe baza studiului efectuat putem concluziona că, în general, la plantele ce cresc în condiții de iluminare mai slabă cantitatea de clorofile totale şi cantitatea de clorofila b este mai mare comparativ cu plantele ce se dezvoltă în condiții de iluminare intensă. Valoarea raportului dintre cantitatea de clorofilă a şi cantitatea de clorofilă b este mai mare la plantele heliofile, deoarece frunzele acestora conțin mai puțină clorofilă b, ceea ce indică faptul că în lumină mai intensă complexul de pigmenți antenari periferici (în care predomină clorofila b) este mai puțin dezvoltat. Determinarea cantităților de clorofilă s-a măsurat la probele provenite de la speciile de plante studiate, cu absorbanțele între 400 şi 700 nm. Pe baza spectrului de absorbție a pigmenților asimilatori se observă diferențele dintre plantele sciafile şi heliofile în ceea ce priveşte cantitățile de pigmenți asimilatori.