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Anatomic Adaptive Strategies of some *Cormophytes* with Individuals Growing in Light and Shade Conditions

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Abstract

The adaptive structural and functional strategies enable plants to survive in variable biotopes. The intensity of some parameters of abiotic factors can be estimated from the environment on the basis of anatomic studies. In this research paper there are histo-anatomical studies of the leaf blades and stems of 10 angiosperm species which have populations growing in both open and shaded places from mountainous biotopes in the Ciuc-Basin¸ with the application of methods for making microscopical preparations. The qualitative evaluation of the histo-anatomical properties was combined with determination of stomata density and biometric data. Up to the present, there have been no histo-anatomical studies in plants distributed in these areas. The studied species possess histological structures adapted to utilizing more efficiently the light energy and humidity accessible to plants in their habitats. The structural adaptations to illumination conditions of the studied species can be distinguished in the structure, types and development rate of the assimilatory and mechanic tissues.

Keywords: adaptive structural particularities, leaf blade, stem, assimilatory tissues, mechanical tissues, mesophyll

Introduction

The study of morphologic properties and internal structures of plant organs according to the variations of environmental factors is important for a better understanding of vital strategies and adaptive structural particularities.

Between the limits permitted by genotype, the structural organization of the leaf adapts very flexibly to the quantity of light and heat and simultaneously, physiologic and functional accommodation reacts to the intensity and duration of fluctuations determined by periodic daily and seasonal alterations (Bercu, 2001; Gonzáles and Gianoli, 2004; Kocsis *et al*., 2004; Lens *et al*., 2004; Mojzes *et al*., 2005; Prusinkiewicz and Rolland-Lagan, 2006; Sieburth and Deyholos, 2006). As final development is installed, no other modifications can be made in the internal structure, even if the illumination conditions are later changed (Tsiantis and Langdale, 1998).

In the structure of cormophyte leaves there are some assimilatory parenchyma types whose morphology and association with other tissues is different because of their different contact with parameters of environmental factors (Deliu, 1993).

Due to the specific architecture, the palisade tissue facilitates the penetration of light in lower leaf layers and its more uniform distribution, while spongy tissue is adapted to the better utilization of the decreased light quantity. According to this structure, the heterogeneous mesophyll increases the efficiency of utilization of incidental light (Bussotti and Grossini, 1997).The organization of the palisade tissue is affected by the intensity of the light: under strong illumination conditions more overlaid layers with long palisade cells are developed, while under shaded conditions this tissue is weakly represented or does not develop at all (György, 2005). Light intensity determines variations in palisade and lacunar parenchyma structures, and the combined effect of light and soil water influences the grade of sclerenchimatization of the leaf and palisade parenchyma consistency (Roãcàas *et al*., 2001; Chunxia *et al*., 2008; Guerfel *et al*., 2009; Sánchez-Azofeifa *et al*., 2009).

Some plant species with individuals growing in different environmental conditions (for example the intensity of light), are more efficiently adapted to the variations of climatic factors. In this thesis we studied comparatively the structural particularities of the above-ground organs of species which have populations growing in both open and shaded places.

Materials and methods

Within the present study, structural features of above-ground organs of 10 angiosperm species were comparatively studied, with individuals spread both in open and in shaded in situ places. The investigated species are the following: *Helleborus purpurascens* W. et K., *Aconitum variegatum* L. (*Ranunculaceae*), *Chamaespartium sagittale* L. (*Fabaceae*), *Galium vernum* Scop. (*Rubiaceae*), *Betonica officinalis* (L.) Trevis, *Ajuga genevensis* L. (*Lamiaceae*), *Digitalis grandiflora* Mill. (*Scrophulariaceae*), *Campanula persicifolia* L. (*Campanulaceae*), *Stellaria holostea* L. (*Caryophyllaceae*) şi *Hypericum perforatum* L. (*Hypericaceae*). The plants derive

from Şumuleu Mic and Şumuleu Mare Mountains¸ situated in the Ciuc-Basin.

The histological study of the organs was carried out in fine sections, obtained from fresh or preserved material, in most of the cases manually (Peacock, 1966; Şerbănescu-Jitariu *et al*., 1983). The colored microscopic preparations were obtained by the simultaneous double staining with Congo red and chrysoidine. Cells and epidermal formations were studied on preparations obtained by epidermis peeling (Fodorpataki, 2001).

The microscopical measurements were made with the help of the objective micrometer and the ocular micrometer. All microphotographs in this thesis are original, taken with a digital camera Canon A250 and a JVC color video camera (CETI type light microscope).

The normality of the data distribution was tested with the Chi-squared test. The distribution of the pooled data was generally not normal, and therefore the non-parametric Mann-Whitney U-test was used. The difference between the stomata number of plants grown in shade and in light 10 species were used with 10 samples for each species, except for the thickness of the palisade parenchym for which 9 species were used with 10 samples for each species. The XLStat software was used for statistical analyses.

Results and discussions

In the case of studied species, the cuticle that covers the epidermis of the leaves is thin or with moderate thickness and no meaningful differences appear in the cuticle thickness between individuals growing under different illumination conditions.

Between epidermal formations, the presence, disposal and types of trichomes are varied: unicellular trichomes on the adaxial epidermis of *Aconitum variegatum* (in reduced number), *Chamaespartium sagittale* and on the abaxial epidermis of *Helleborus purpurascens* and *Galium vernum*; multicellular trichomes on both of the epidermis of *Betonica officinalis*, *Ajuga genevensis*, *Digitalis grandiflora*; uni- and multicellular trichomes on both sides of the leaf at *Stellaria holostea*. Generally the trichomes are present in a low number. The leaves of *Campanula persicifolia* and *Hypericum perforatum* are glabrous.

By the position of the stomata, the leaves of *Chamaespartium sagittale, Betonica officinalis, Ajuga genevensis, Digitalis grandiflora, Stellaria holostea* are amfistomatic, and those of *Helleborus purpurascens, Aconitum variegatum, Galium vernum* and *Hypericum perforatum* are hypostomatic. The number of stomata at individuals living under intense luminosity conditions is higher, compared to those deriving from areas where the intensity and quantity of accessible light for plants is low (Tab. 1). At *Campanula persicifolia* leaves of individuals collected from places exposed to light (mountain hay fields) are amfistomatic, while leaves of individuals growing under shaded conditions (under trees from the margin of forests) are hypostomatic; and in both cases the number of stomata are approximately equal. Amphistomaty and leaf compartmentation have been evaluated concerning leaf xeromorphy (Yiotis *et al*., 2006). At the majority of the species on the surface of the leaf blade the stomata are disposed on the level of the epidermis, while at *Aconitum variegatum, Betonica officinalis* and *Ajuga genevensis* the stomata are slightly observable towards the level of the epidermis.

The adaxial epidermis of *Campanula persicifolia* compared to the abaxial side is composed of larger cells, having the function of water storage (Fig. 1.). Similar situations can be observed at species like *Helleborus purpurascens, Aconitum variegatum, Galium vernum* and *Ajuga genevensis.* In the case of the same species, the anticlinal walls of the epidermic cells in leaves of individuals growing in the shadow are undulated, on the other hand at species that vegetate under direct light effect, the adaxial epidermis is straight, while the abaxial is undulated. Exceptions make the leaves of *Campanula persicifolia*, where the epidermis is not presenting undulated cell walls in either of the cases. The leaves of the studied plants are bifacial, in most of the cases with dorsiventral structure (exception *Digitalis grandiflora* with homogenous mesophyll in case of individuals living at the edge of forests, under shaded conditions). On the basis of biometric data, generally the leaves of plants from places exposed to light are thicker (Tab. 2).

Leaves at low light usually present an increased assimilate investment in leaf size to improve light interception, whereas at high light leaves are comparatively thicker, with a high leaf dry mass per area (Gonçalves *et al*., 2008). At different species, the length of the palisade cells is varied and even within the same species under different living conditions there are differences, or the length of these cells decreases towards the margin of the assimilatory organ (Wilkinson, 1994; Aranda *et al*., 2004; Konoplyova

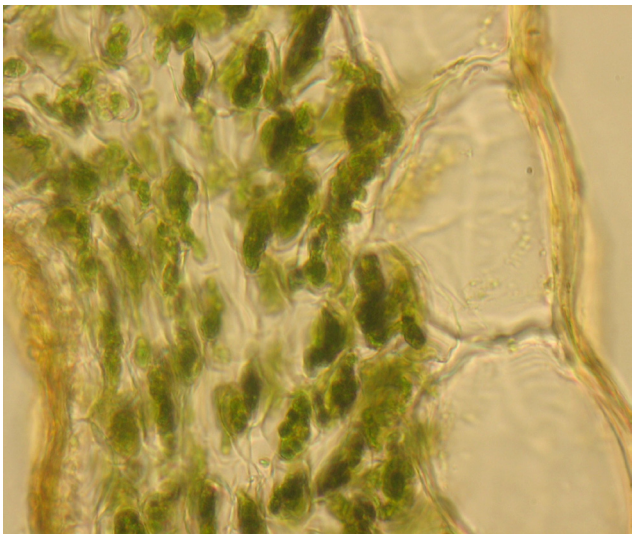

Fig.1. The internal structure of the *Campanula persicifolia* leaf, with thick epidermal cells (600x)

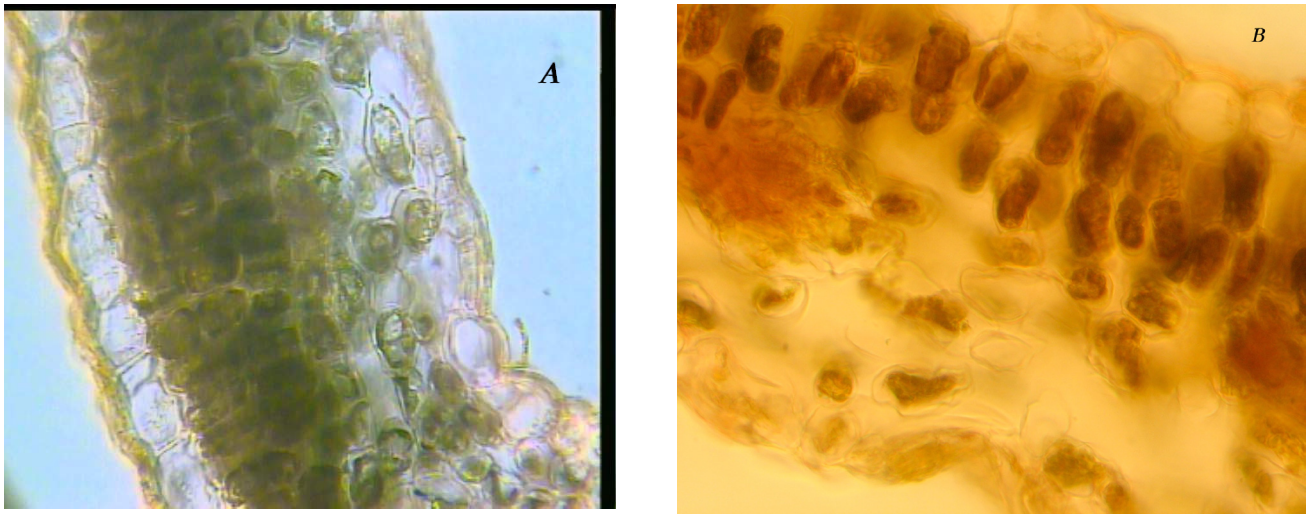

Fig. 2. The structure of the leaf of *Ajuga genevensis* under direct light effect (A) and in shadow (B) (600x)

et al., 2008). The structure, type and development of the assimilatory tissues are the structural adaptations of these plants against the quantitative and qualitative differences in light. In *Helleborus purpurascens, Betonica officinalis*, *Ajuga genevensis, Aconitum variegatum* and *Galium vernum* there are more pronounced differences in the development of the palisade parenchyma (Fig. 2). Only in the case of *Helleborus purpurascens* the lacunose assimilatory tissue is richer in intercellular spaces in case of individuals living in shadow. In these species, palisade parenchyma represents a higher percentage of mesophyll, the cells are more stretched or the palisade parenchyma is built up with more cell layers in the individuals grown in open places (example: *Betonica officinalis, Galium vernum*).

The effect of light is more evident in *Digitalis grandiflora*: individuals grown in mountains hayfields have heterogeneous mesophyll (with assimilatory tissue differentiated in palisade and spongy parenchyma). By contrast, in the individuals occurring at the edges of forests in the shadow the mesophyll is homogenous (constructed exclusively of spongy parenchyma, the first two layers under the adaxial epidermis containing more chloroplasts).

Differences in assimilatory tissue structure are present in the case of *Campanula persicifolia,* too. When the plant is exposed to direct solar light, in the mesophyll of the leaf blade both palisade (formed of 1-2 cell layers) and spongy parenchyma (rich in intercellular spaces) are developed. Under lower light conditions, the mesophyll of the leaf near the principal nervure area is homogenous, while on other areas the palisade parenchyma is slightly developed and the spongy parenchyma contains less intercellular spaces.

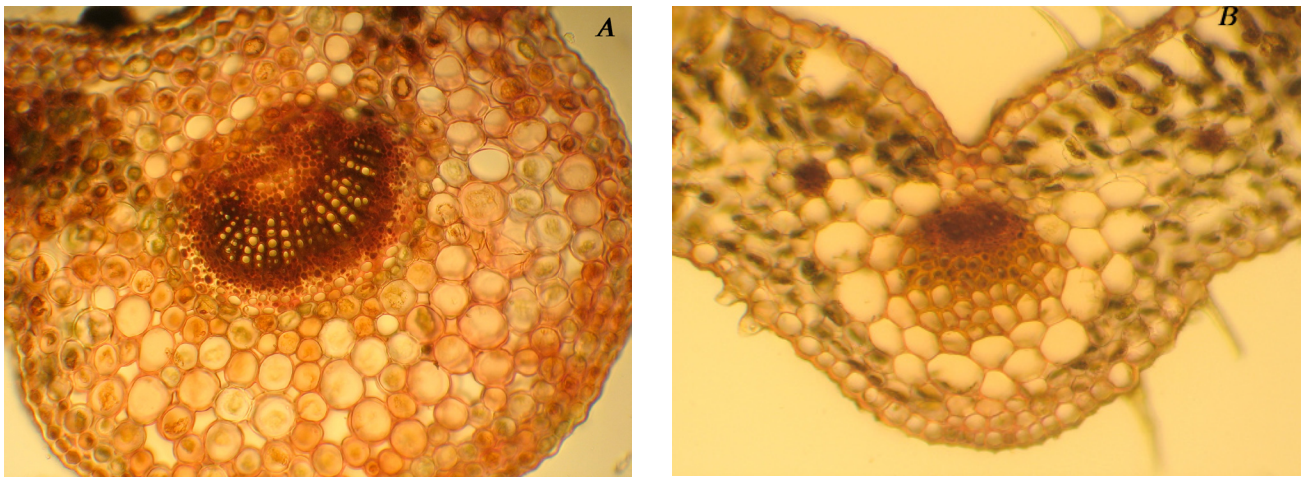

Fig. 3. Aquiferous cells in the structure of the principal nervure of *Betonica officinalis* (A) and *Stellaria holostea* (B) (150x)

The structure of the leaf blade in *Stellaria holostea*, *Hypericum perforatum* and *Chamaespartium sagittale*, grown in open places and in shaded ones is similar.

The density of venation and the size of the vascular bundles vary in the different species. We cannot observe significant differences in the development of the vascular

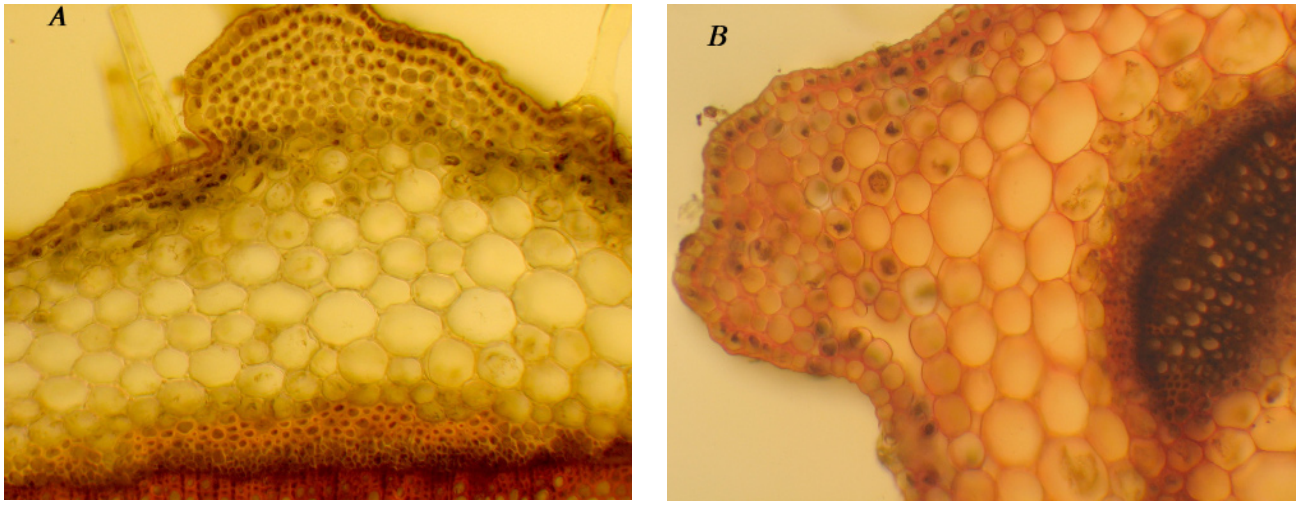

Fig. 4. The structure of the stem angles of *Ajuga genevensis* grown in direct light effect (A) and in shadow (B) (150x)

tissue in leafs deriving from individuals spread on differently illuminated places. At most of the studied plants in the composition of the vascular bundles the xylem is more represented. In the structure of *Stellaria holostea, Chamaespartium sagittale* and *Galium vernum* the development of the xylem and the phloem is approximately equal. In the case of some species in the parenchyma of the principal nervure aquiferous cells can be found, too (for example *Stellaria holostea, Betonica officinalis, Ajuga genevensis,*

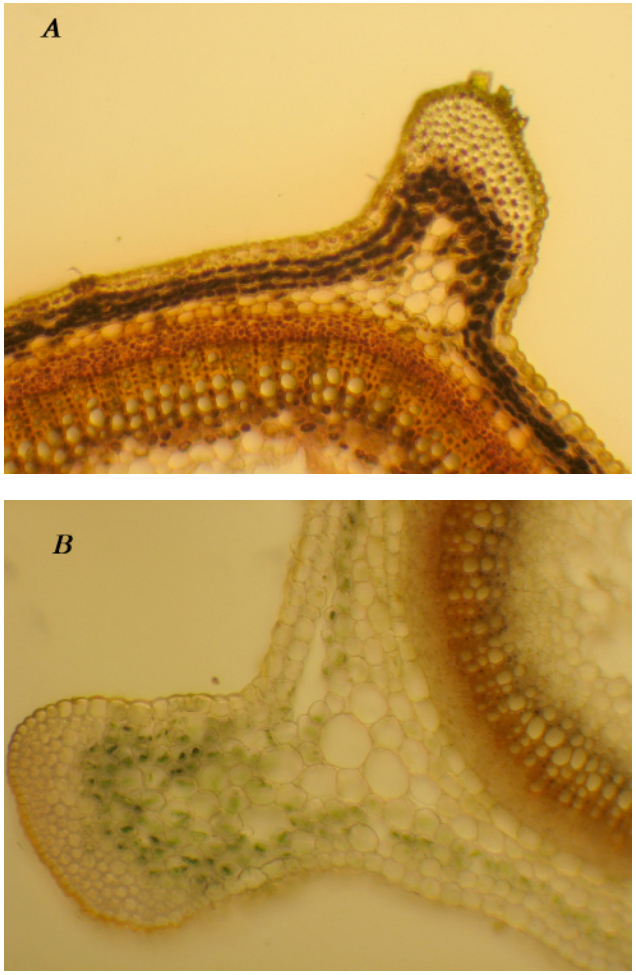

Fig. 5. Stem structure of *Galium vernum* under direct light effect (A) and in shadow (B) (150x)

Tab. 1. The number of stomata per mm^{-2} leaf blade, in case of plants grown in places with different illumination conditions (average ± S.D. (standard deviation), n = 10)

Species	In intense lightened places		In diffuse lightened places	
	Adaxial epidermis	Abaxial epidermis	Adaxial epidermis	Abaxial epidermis
Helleborus purpurascens W. et K.	0	70±10.19	0	31±10.76
Aconitum variegatum L.	0	94±17.65	0	74±9.22
Cham aespartium sagittale L.	110±18.07	60±11.67	33±9.98	69±18.28
Galium vernum Scop.	0	109±17.95	0	58±12.87
Betonica officinalis (L.) Trevis	44±11.68	152±20.36	9±3.38	64±21.90
Ajuga genevensis L.	38±16.19	122±38.08	19±9.17	66±15.21
Digitalis grandiflora Mill.	53±14.78	180±13.38	28±16.57	137±11.14
Campanula persicifolia L.	12±7.23	184±26.64	0	194±27.81
Stellaria holostea L.	47±7.23	21±7.38	26±7.17	15±8.35
Hypericum perforatum L.	0	159 ± 38.41	0	138±12.10

Tab. 2. Biometric data of plant leaves grown in environments with different light intensity (average ± S.D., n = 10)

Species	Leaf blade thickness (µm)		Mesophyll thickness (µm)		Palisade parenchyma thickness (µm)		Percentage of palisade parenchyma in the mesophyll (%)	
	In open land	In shaded spaces	In open land	In shaded spaces	In open land	In shaded spaces	In open land	In shaded spaces
Helleborus purpurascens W. *et*. K.	306.54±26.95	223.72±20.93	233.24±29.51	166.12±18.56	86.63±8.63	51.88±3.51	37.14±5.39	31.23±3.53
Aconitum variegatum L.	347.96±20.83	229.12±26.88	270.84±21.10	175.92±21.78	96.15±6.66	50.26±11.51	35.50±3.24	28.57±4.89
Chamaespartium sagittale L.	202.03±10.78	183 ±13.06	158.6 ±13.11	144.20±11.92	57.34±7.21	44.90±5.42	36.15±5.47	31.13±4.13
Galium vernum Scop.	163.48±11.95	93.94±5.78	125.42±14.65	60.27±7.72	55.39±8.76	23.91±4.71	44.16±5.39	39.67±7.38
Betonica officinalis (L.) Trevis	191.05±7.19	168.60±13.49	141.76±7.67	123.46±10.80	71.25±16.54	40.50±5.17	52.37±10.82	32.80±2.91
Ajuga genevensis L.	193.74±10.68	191.54±18.87	140.79±14.64	135.42±18.52	70.27±11.14	54.90±6.11	49.91±5.20	40.54±7.15
Digitalis grandiflora Mill.	182.02±12.87	248.15±11.79	146.64±11.54	198.86±13.52	42.7±5.30	0	29.12±3.06	0
Campanula persicifolia L.	235.46±12.67	205.45±21.42	167.87±12.43	144.69±13.02	75.15±13.79	40.99±8.97	44.76±8.11	28.33±4.89
Stellaria holostea L.	194.22±16.11	234.73±16.54	147.86±12.24	186.66±16.91	42.70±6.43	45.63±15.38	28.87±5.39	24.44±6.56
Hypericum perforatum L.	159±6.83	111.75±6.06	106.87±7.61	75.40±3.72	50.99±4.06	44.16±7.03	47.71±9	58.57±8.65

Campanula persicifolia and *Hypericum perforatum*) (Fig. 3).

In the structure of *Hypericum perforatum, Campanula persicifolia, Aconitum variegatum, Galium vernum* and *Digitalis grandiflora* leaves the mechanical tissue is missing.

Tab. 3. Data concerning mechanical tissues in the area of the principal nervure of some of the plant leaves. Number of cells in cross sections (average ± S.D., n = 10)

Species	Number of collenchyma cells		Number of sclerenchyma cells	
	In open land	In shaded spaces	In open land	In shaded spaces
Helleborus purpurascens W. et K.	0	0	51±3.97	31±3.43
Chamaespartium sagittale L.	0	9±2.40	9±1.56	0
Betonica officinalis (L.) Trevis	85±5.94	0	0	0
Ajuga genevensis L.	50±8.71	35±3.86	0	0
Stellaria holostea L.	0	0	32±2.83	45±6.16

At *Stellaria holostea* near the phloem of the vascular bundle there is a belt of sclerenchyma. In the case of *Helleborus purpurascens* the sclerenchyma surrounds the vascular bundles and in the case of leaves of *Ajuga genevensis* there is collenchyma around the phloem. In the case of some of the species mentioned above, significant differences exist in the grade of mechanical tissue development depending on the habitat of the phyto-individuals. Under direct illumination conditions, in leaves of *Chamaespartium sagittale*, beneath the phloem of the vascular bundles there are some sclerenchyma cells, while in individuals grown under shadow, some cells of collenchyma are formed (Tab. 3.). Under shaded conditions, leaves of *Betonica officinalis* do not possess mechanical tissue, while leaves of samples living on mountain hayfields present collenchyma surrounding the phloem part of the vascular bundles.

In the mesophyll of the leaves of *Stellaria holostea* some cells with calcium oxalate druses can be found, respectively at *Hypericum perforatum* secretory canals can be observed.

There are significant differences in the number of stomata between individuals grown in open places and in shaded conditions (Mann-Whitney U-test, $p<0.001$). For the 10 plant species involved in the analysis, statistically significant differences were found between individuals

growing in illuminated and shaded conditions as concerning the height of the palisade parenchyma (Mann-Whitney U-test, $p<0.001$). No difference was found concerning the thickness of the leaf blade (Mann-Whitney U-test, $p=0.84$) and thickness of mesophyll (Mann-Whitney U-test, $p=0.34$).

Only three of the 10 species studied living in places with different light conditions presented differences considering internal stem structure and these are connected to the development of mechanical tissue. Collenchyma from the angles of stems, a general feature of *Ajuga genevensis*, does not develop in shaded conditions (Fig. 4). In the case of *Chamaespartium sagittale* and *Galium vernum* in direct light the mechanical tissue of stems is represented by sclerenchyma, but in shaded conditions by collenchyma.

Well-developed assimilatory tissue appears in the internal stem structure of *Helleborus purpurascens* (relatively thick cortex is represented by clorenchyma) and *Chamaespartium sagittale* (in the cortex, as well as in the prolongation of the winged stem, tissues similar to homogenous mesophyll are developed). At other species (*Betonica officinalis, Galium vernum, Aconitum variegatum* and *Stellaria holostea*) the greatest part of the structure of the relatively thin cortex is occupied by clorenchyma (3-7 cell layers) (Fig 5). In the stem structure of *Digitalis grandiflora, Ajuga genevensis* and *Hypericum perforatum* the assimilatory parenchyma is weakly represented (1-2 cell layers on the external part of the cortex).

The vascular system in the stem structure of all of the 10 studied species is well represented. At some of the species this system is composed of vascular bundles disposed on a single circle, at others (*Digitalis grandiflora, Chamaespartium sagittale, Hypericum perforatum, Galium vernum*) the stem is *Tilia* type. In the structure of the stem of Stellaria holostea the xylem and the phloem in the vascular bundles are approximately equally developed. At the other species the xylem that takes part in the composition of the vascular bundles is more developed than the phloem.

The medular parenchyma is developed at all of the ten species mentioned above. In the structure of *Aconitum variegatum* the cells are full of stored substances. The epidermis of stems of *Betonica officinalis* and *Stellaria holostea* present multicellular trichomes

Conclusions

The influence of the light in the structure of the leaf at species collected both from directly illuminated and from shaded places, appears differentially depending on species in structure, differentiation and development of the assimilatory tissue. Generally, the number of stomata is higher in plant individuals living in strongly illuminated conditions, as compared to those growing is stands with reduced light intensity. On the other hand, differences exist in some species concerning the development and presence or absence of mechanical tissue. Only in the case of some species can differences be observed in the internal structure of the stem between structural characteristics of the individuals depending on the grade of illumination. These differences can be observed on the level of the mechanical tissue.

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