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Functional Anatomy of Leaf *Campanula alliariifolia* Willd.

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Abstract

The functional anatomy of the leaf of *Campanula alliariifolia* Willd. is examined. The purpose of this research is to identify features of the functional anatomy of the leaf of this species in order to understand the mechanism of its adaptation to new growth conditions. Plant material of research are plants of *Campanula alliariifolia* Willd. in: a) their natural growth habitat (the Caucasus, city Sochi) and b) the region of introduction (the steppe zone of Ukraine, city Donetsk). The research methods used are the standard techniques of anatomical research. In the region of introduction, the research reveals the formation of columnar mesophyll and stomata on the adaxial side of the leaf of *C. alliariifolia*; increase in mesophyll thickness, stomata frequency and size, and in the moisture content. The rosellate leaf area and the cauline leaf dry matter accumulation are greater than in the natural growth habitat. The main ways of adaptation are :a) qualitative and quantitative anatomical changes in leaves; b) correlation of changes of the majority of anatomical and morphological parameters; c) identical physiological and anatomical changes in leaves of both formations.

Keywords: adaptation, *Campanula*, leaf, steppe zone of Ukraine

Introduction

The habitat influences the genotype of organisms, which responds to changes in the conditions of existence. Leaf structure reflects the environmental evolution of species, which is shaped by past and present changes in environmental conditions (Dyachenko, 1978; Goryshina *et. al*., 1992; Kul'tiasov, 1963; Poplavskaya, 1937; Schwartz, 1980; Serebryakov, 1952; Shennikov, 1950). Therefore, information about the leaf structure allows to expand the environmental characteristics of the species, clarify current knowledge of the structure variability range of the photosynthetic apparatus and of the amplitude of its plasticity. This knowledge is the key to learn the adaptive possibilities of plants under the conditions of introduction, breeding and selection for resistance of species.

Adaptation of plants to extreme environmental conditions is associated with significant restructuring of their assimilating system, which is highly sensitive to external influences. The photosynthetic apparatus of plants has high plasticity, which results in significant variability of leaf size and its anatomical characteristics (Lukyanova *et. al*., 1999; Nikcholaevskaya, 1990). The adaptation process involves the whole organism. Morphological and anatomical reconstructions linked to this process change physiological status of the plant. As a result, the plant is able to use the resources of environment in new extreme conditions of introduction more effectively (Menshakova *et. al*., 2008). The activity of various adaptive mechanisms of plants is directed towards reduction of impact of damaging factor to subliminal values (Crawford, 1989).

Morphological and physiological features of a plant leaf are linked to the nature of its functioning. When habitat conditions of the plant change, these features also change. Research of the functional morphology of leaves of plant species in the conditions of their region of introduction allows to identify the level of their adaptation, to provide scientific basis for their cultivation and reproduction to add them to the plant range for landscape design in the conditions of the steppe zone of Ukraine.

The majority of types of *Campanula L*. (bell-flower) species, including *Campanula alliariifolia* Willd., are decorative. Some of them were introduced and became part of floriculture some time ago. In addition, many types of this species are rare and need protection. Therefore, it is important to preserve genofunds of these species under ex situ conditions in regions of introduction with their possible subsequent reintroduction in natural ecotops.

The purpose of the present study is to analyse the characteristics of functional anatomy of the leaf of *C. alliariifolia* in order to understand the mechanism of adaptation of this species to the conditions of the steppe zone of Ukraine.

Material and methods

Generative plants of *Campanula alliariifolia* Willd. were sampled from their natural habitat (city Sochi: limestone cliffs and forest), introduction region (the steppe zone of Ukraine: sunny area and shaded area) and from a greenhouse. Morphological features of leaves of 20 specimens from each location were examined: leaf shape; features of leaf edge, the crown, the bottom; linear dimensions and the area of leaf plate; the diameter and length of the petiole, and leaf weight. Morphological characteristics of leaf were determined by using classification outlined in the "Ecoflora of Ukraine" (2000). Leaf plate and petiole sections were made in the middle part of the plant. Thickness of leaves, the epidermis and of the mesophyll was measured at the same distance from the leaf edge and from the main vein. Cross-sectional area of petiole, parenchyma and of the vascular and strengthening tissues were determined.

A Zeizz Primo Star microscope was used during research; AxioVision software was used for measurements.

The method of microreplication (Klein, 1974) was applied to make preparations of the epidermis of leaves. The classification of Zakharevich (1954) was used to characterize the outlines and projections of epidermal cells, while the morphological classification of Baranova (1985) was applied to characterize the stomata apparatus. The structure of *C. alliariifolia* rosellate leaf from a natural habitat (city Sochi: limestone cliffs and forest) was compared with different growth conditions in the region of introduction: a) sunny area, b) shaded area and c) greenhouse. The average relative outdoor illuminance (at noon on a clear day) during the vegetation period was 100%, 15.2%, 3.3%, respectively as per above-mentioned growth conditions in the region of introduction. Comparison of climatic factors of research regions (city Donetsk, Ukraine; city Sochi, Russia) is shown in Fig. 1.

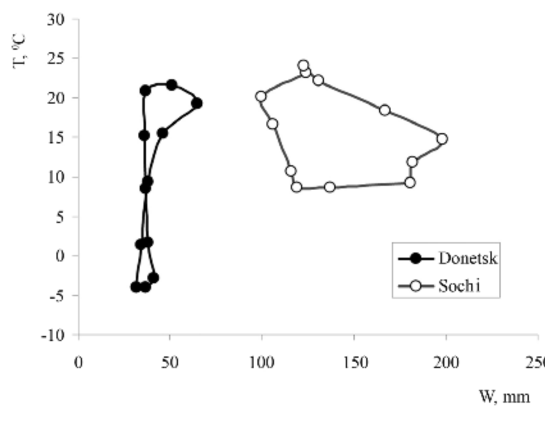

Fig. 1. Climagram of research regions: T, °C – average monthly temperature, °C; W, mm – amount of precipitation, mm

Results and discussion

Under the conditions of the steppe zone of Ukraine, in both sunny and shaded area, rosellate leaf length of *C. alliariifolia* increased respectively by 1.5 and 1.4 times (Tab. 1). The width of leaf was not significantly different, and the index of leaf declined by 0.77 and 0.66 times, respectively.

Tab. 1. Leaf morphometric parameters of *Campanula alliariifolia* Willd.

Group	Green weight, g		Dry weight, g		Leaf plate size			Petiole size	
	leaf	leaf plate	leaf	leaf plate	length, cm	width, cm	area, cm^2	length, cm	diameter, mm
a	0.684± 0.14	0.475± 0.11	0.130± 0.03	0.092± 0.02	4.1± 0.48	4.6± 0.53	15.9± 3.65	5.8± 0.54	–
b	1.064± 0.07^{**}	0.813± 0.06^{**}	0.139± 0.02	0.099± 0.01	6.1± 0.28^{**}	5.3± 0.24	20.9± 1.58	6.4± 0.34	1.59± 0.08
c	1.106± 0.05^{**}	0.861± 0.04^{**}	0.183± 0.011	0.141± 0.009^{*}	5.6± 0.22^{**}	6.8± 0.31	25.9± 1.81^{*}	6.8± 0.31	1.44± 0.05
d	0.251± 0.02^{**}	0.184± 0.02^{**}	0.043± 0.004^{**}	0.031± 0.003^{**}	3.5± 0.17	3.7± 0.12	10.0± 0.75	5.6± 0.35	0.75± 0.06
e	0.149± 0.02	0.129± 0.02	0.029± 0.004	0.025± 0.003	3.5± 0.26	3.37± 0.26	8.34± 0.99	1.58± 0.24	–
f	0.165± 0.02	–	0.027± 0.003^{**}	–	2.9± 0.12^{*}	2.2± 0.15^{**}	4.1± 0.47^{**}	0.4± 0.06^{**}	–
g	0.202± 0.03	–	0.028± 0.01^{**}	–	3.2± 0.26	2.9± 0.30	7.0± 1.35	0.4± 0.06^{**}	–

Notes. * – the difference of average measures is significant when P> 0.95; ** – when P> 0.99; Group – conditions of growth / leaf formation; a – Sochi city, river Khosta, forest, limestone cliffs / rosellate; b – DBG, sunny area / rosellate, c – DBG, shaded area/rosellate; d – greenhouse / rosellate; e – Sochi city, river Khosta, forest, limestone cliffs / cauline; f – DBG, sunny area /cauline; g – DBG, shaded area / cauline; DBG – Donetsk Botanic Garden of Academy of Sciences of Ukraine

Significant difference in the leaf plate area was observed only in plants in a shaded area (1.6 times greater than in the natural habitat). Green leaf weight (M) and the leaf plate weight (m) in the experiment (sunny and shaded area) were respectively 1.6, 1.7 and 1.7, 1.8 times greater. In the greenhouse, M and m have changed by 0.36 and 0.27 times. The dry leaf plate weight (m1) in a shaded area was 1.5 times greater than in the natural habitat. In a sunny area, the ratio of the leaf plate green weight to its area (m1/s) significantly increased (by 1.3 times), while the ratio of the leaf dry weight to the area (M1/s), the ratio of the leaf dry weight to the leaf green weight (M1/M) and the ratio of the leaf plate dry weight to the weight of the green leaf (m1/m) decreased by 0.78, 0.65 and 0.60 times, respectively. In a shaded area, there was a significant decrease (by 0.8 times) in the ratio of leaf dry weight to the leaf green weight (M1/M).

Based on the comparison of the above mentioned parameters in the sunny and shaded areas, a 0.83-fold decrease of the leaf plate area of *C. alliariifolia* was observed in a sunny area. Green weight of the leaf and of the leaf plate had no significant differences. Dry weight of the leaf and of the leaf plate in a sunny area was respectively 0.76 and 0.70 times smaller. There was a significant difference in the leaf indexes: in a sunny area, the ratio of the leaf plate weight to the area (m/s) and the ratio of the leaf weight to the leaf plate area (M/s) were 1.2 times greater, and the ratio of the leaf plate dry weight to its area (m1/s) declined by 0.80 times. The ratio of the leaf dry weight to the green one (M1/M) and the ratio of the leaf plate dry weight to the green one (m1/m) were 0.80 times smaller.

In a sunny area of the region of introduction, *C. alliariifolia* cauline leaves are characterized by lower values of leaf plate area (by 2.0 times), of leaf size (by 1.2-1.5 times), and of leaf index (by 1.2 times) compared to the natural habitat. The leaf green weight in the experiment (in both sunny and shaded areas) was not significantly different from the control value, but the ratio of the leaf green weight to the leaf plate area (M/s) increased by 2.5 and 1.97 times, respectively. In the sunny and shaded areas of the region of introduction, the leaf dry weight (M1) decreased by 0.93 and 0.97 times, respectively, while the ratio of the leaf dry weight to its area (M1) grew respectively by 1.89 and 1.08 times.

Comparison of the cauline leaves of plants in the sunny and shaded areas revealed differences only in three parameters. In a sunny area, there was an increase in ratios of a) the leaf weight to the leaf plate area (M/s) – by 1.31 times, b) the leaf dry weight to the leaf plate area (M1/s) - by 1.75 times, and c) the leaf dry weight to the green one (M1/M) – by 2.0 times.

Comparison of *C. alliariifolia* leaf plate anatomical characteristics with those in the natural habitat revealed a differentiation in mesophyll, which appeared to be columnar and spongy, in rosellate and cauline leaves under all experiment conditions (sunny area, shaded area, and greenhouse) (Fig. 2 and 3). Rosellate leaf thickness in the experiment grew by 1.3-2.0 times, the thickness of mesophyll – by 1.4-2.3 times (Tab. 2).

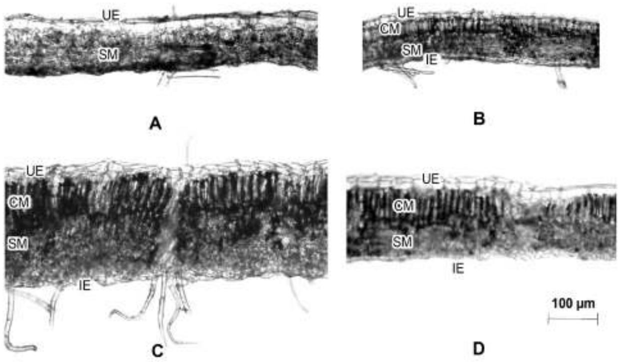

Fig. 2. Features of rosellate leaf anatomical structure of *Campanula alliariifolia* Willd.: A – in the natural habitat (city Sochi, river Khosta, forest, limestone cliffs), B – in the greenhouse, C – in a sunny area in the steppe zone of Ukraine, D – in a shaded area in the steppe zone of Ukraine: ue – the upper epidermis, ie – the inferior epidermis, cm – columnar mesophyll, sm – spongy mesophyll

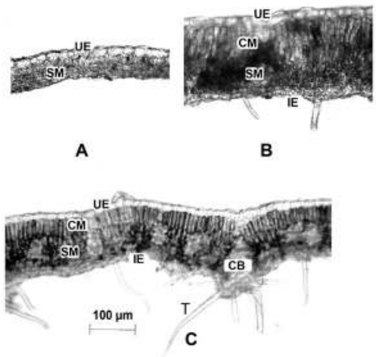

Fig. 3. Anatomical features of *Campanula alliariifolia* Willd. cauline leaf: A – in the natural habitat (city Sochi, river Khosta, forest, limestone cliffs), B – in a sunny area in the steppe zone of Ukraine, C – in a shaded area in the steppe zone of Ukraine: ue – the upper epidermis, ie – the inferior epidermis, cm – columnar mesophyll, sm – spongy mesophyll, cb – conducting bundle, t – trichome

Tab. 2. Anatomical indicators of leaf plate species of *Campanula alliariifolia* Willd.

Group	Leaf plate thickness, µm	Mesophyll thickness, µm			Thickness epidermis, µm	
		total	palisade	spongy	adaksial side	abaksial side
a	104.9± 6.11	75.8± 4.63	mesophyll undifferentiated	–	23.9± 1.44	11.6± 1.26
b	210.1± 7.67**	176.3± 7.32**	75.1±3.16	100.7± 6.32	22.0± 1.39	13.5± 0.73
c	158.5± 6.78**	128.1± 6.10**	59.9±3.79	66.6± 4.32	19.6± 1.10	14.7± 1.23
d	136.6± 7.59*	107.7± 6.46**	54.0±4.17	52.8± 3.23	17.0± 1.72*	11.0± 1.05
e	87.13± 2.74	57.79± 2.66	mesophyll undifferentiated	–	16.71± 0.34	9.01± 0.83
f	222.0± 7.27**	189.2± 7.63**	92.4± 6.25	96.4± 6.55	19.1± 0.85*	12.4± 0.89*
g	137.2± 6.67**	108.8± 5.61**	52.9± 3.81	55.4± 4.43	18.1± 1.15	11.8± 0.70

Notes. * – the difference of average measures is significant when P> 0.95; ** – when P> 0.99; group – conditions of growth / leaf formation; a – city Sochi, river Khosta, forest, limestone cliffs / rosellate; b – DBG, sunny area / rosellate, c – DBG, shaded area/rosellate; d – greenhouse / rosellate; e – city Sochi, river Khosta, forest, limestone cliffs / cauline; f – DBG, sunny area /cauline; g – DBG, shaded area / cauline; DBG – Donetsk Botanic Garden of Academy of Sciences of Ukraine

The formation of columnar mesophyll, the increase in the number of the palisade tissue layers, and the increase in their height are specific xeromorphic characteristics of mesophytes in arid growth conditions. It is known that growth in arid environments and under high solar radiation leads to greater leaf plate thickness and assimilative tissue (Dyachenko, 1978; Poplavskaya, 1937; Vasilevskaya, 1938, 1954). The increase in the number of layers and in thickness of palisade mesophyll has adaptive character because the main leaf photosynthetic activity is connected in particular with palisade tissue. This is due to the fact that the new growth of chloroplasts in palisade tissue is much longer (Mokronosov *et al*., 1973) and, consequently, the number of chloroplasts in the palisade tissue cells is 2-3 folds higher than in other photosynthetic tissues. The ultrastructure of plastids in palisade cells is notable for strong development and has a strong thylakoids induration, whereas in spongy tissue chloroplasts have a more friable structure. This is due to the fact that in the leaf ontogeny the differentiation of spongy tissue ends early, whereas plastid complex in palisade tissue is being formed while the leaf is growing, synchronously with the increase in photosynthesis and in the Ribulose-bisphosphate carboxylase (RuBisCO) activity. For palisade tissue, a higher level of protein synthesis is typical, which means cells growth and new growth of plastids during the entire period of plant growth. In contrast, in spongy tissue these processes terminate early. The high degree of specialization of palisade tissue, which can perform the function of photosynthesis, is also evident in its connection with the intercellular spaces system. Per unit of leaf tissue volume, the cells surface of palisade parenchyma facing the intercellular spaces exceeds that of the spongy parenchyma cells by 1.6-3.5 times (Esau, 1980). Due to relatively heavy flow of the sucrose synthesis, spongy parenchyma is specialized in transportation of assimilants from the leaf more than palisade parenchym; starch is mainly synthesized in palisade parenchyma (Mokronosov *et al*., 1973).

In the sunny and shaded areas of the region of introduction, the thickness of the cauline leaf was 1.6-2.5-fold greater, and mesophyll thickness increased by 1.9 and 3.3 times. One way of plant adaptation to high solar radiation and dry environmental conditions was reduction of the size of leaf plate and a simultaneous increase in its thickness. *C. alliariifolia* cauline leaves in a sunny area in the region of introduction increased significantly in the thickness of adaxial epidermis (by 1.2 times) and of abaxial one (by 1.4 times) compared to the natural habitat. Epidermal cells of *Campanula* species perform the function of water storage (Gyorgy, 2009), thus the increase in thickness is an adaptive response to the lack of moisture in habitat.

Comparison of anatomical parameters of rosellate and cauline leaves in the sunny and shaded areas in the region of introduction showed that the thickness of the leaf, in particular columnar and spongy mesophyll, is significantly greater in a sunny area. Gyorgy (2009) discovered that a layer of palisade tissue was formed in the leaves of *C. persicifolia* under good illumination conditions. This layer was absent in the plants of a shaded growth place.

The study of anatomical parameters of *C. alliariifolia* rosellate leaf petiole under conditions of introduction showed that the area of the petiole cross section in a sunny area was 1.1 times greater, the parenchyma area in a cross section was 1.3 times greater, the ratio of parenchyma area to the petiole cross-section area was 1.2 times greater, and collenchyma area ratio to the petiole cross section was 0.8 times smaller than in a shaded area (Tab. 3). As opposed to plants in a shaded area, the ratio of petiole tissues of the plants in a sunny area changed: parenchyma area increased due to reduced collenchyma and vascular tissue. Compared with greenhouse plants, plants from a sunny area had greater area of vascular tissue and parenchyma and a smaller collenchyma area.

Tab. 3. Anatomical indicators of petiole of rosellate leaf of *Campanula alliariifolia* Willd.

Growth conditions	Petiole collenchyma thickness, μm	Cross-section area, mm^2	Tissue area in cross section, mm^2			Conducting bundle area, mm^2
			collen-chyma	paren-chyma	conductive	
DBG, sunny area / rosellate	56.16± 3.36	2.14± 0.21	0.36± 0.03	1.24± 0.13	0.38± 0.03	0.54± 0.05
DBG, shaded area / rosellate	68.39± 5.89	1.88± 0.09	0.39± 0.03	0.93± 0.05*	0.36± 0.02	0.57± 0.03
Greenhouse / rosellate	37.78± 3.04**	0.71± 0.03**	0.16± 0.04**	0.41± 0.05**	0.11± 0.01**	0.14± 0.01**

Notes. * – the difference of average measures is significant when P> 0.95; ** – when P> 0.99; plants growing in a shaded area and in greenhouse were compared to plants growing in a sunny area; DBG – Donetsk Botanical Garden of Academy of Sciences of Ukraine

The plants in a shaded area compared with those from the greenhouse had an increase in vascular tissues due to reduced parenchyma with the same collenchyma area. Consequently, the transfer of plants into open ground (sunny and shaded areas) from the greenhouse stimulates growth of petiole conductive tissue area of rosellate leaf along with an increase in the area of the leaf plate and in its weight. The transfer of plants into high illumination conditions (a sunny area) encourages an increase in the petiole parenchyma area. The leaf weight was not significantly different in the sunny and shaded areas. Under conditions of introduction in the sunny and shaded areas, 3 layers of collenchyma were observed in contrast to 2 layers of strengthening tissue in rosellate leaf petioles of *C. alliariifolia* plant in the natural habitat (Fig. 4).

In the steppe zone of Ukraine, the emergence of stomata on the adaxial epidermis of rosellate and cauline leaves of *C. alliariifolia* was observed, as well as a significant increase in stomata quantity on the abaxial epidermis of the rosellate leaf (by 1.7 times in a sunny area, and by 1.9 times in a shaded area), compared to the natural habitat (Tab. 4).

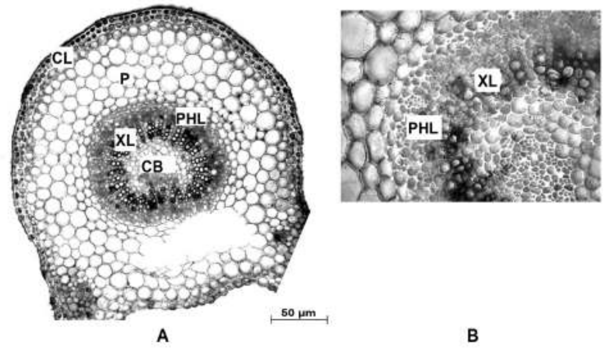

Fig. 4. Anatomical structure of the petiole in the middle part of *Campanula alliariifolia* Willd. rosellate leaf in the steppe zone of Ukraine: cb – conducting bundle, p – parenchyma, cl – collenchyma, xl – xylem, phl – phloem; A – overview of petiole, B – enlarged view of conducting bundle

Tab. 4. Anatomical indicators of abaxial epidermis of the leaf of *Campanula alliariifolia* Willd.

Growth conditions	Quantity per 1 mm², pcs.		Stomata size, µm	
	cells	stomata	length	width
City Sochi, river Khosta, forest, limestone cliffs / rosellate	20.2± 1.55	3.1± 0.34	23.4± 1.29	13.8± 0.28
DBG, sunny area / rosellate	21.1± 0.79	5.2± 0.57*	23.8± 0.59	15.3± 0.86
DBG, shaded area / rosellate	21.3± 1.58	6.0± 0.52**	22.7± 0.87**	15.6± 0.41
Greenhouse / rosellate	23.3± 2.10	4.1± 0.44	22.6± 0.88	14.2± 0.49
City Sochi, river Khosta, forest, limestone cliffs / cauline	19.28± 1.02	4.02± 0.21	21.72± 0.52	13.09± 0.34
DBG, sunny area / cauline	25.9± 2.05**	5.7± 0.48**	19.8± 0.75	14.7± 0.36*
DBG, shaded area / cauline	32.1± 2.02**	6.8± 0.40**	18.8± 0.64*	13.5± 0.45

Notes. * – the difference of average measures is significant when P> 0.95; ** – when P> 0.99; DBG – Donetsk Botanic Garden of Academy of Sciences of Ukraine

In comparison with the control values, the width increased by 1.1 times and the stomata area - by1.6 times. In conditions of the steppe zone of Ukraine, there is a growth in the number of cells and stomata on the abaxial epidermis of cauline leaves compared to the plants from the natural habitat: respectively by 1.4 and 1.3 times in a sunny are, and by 1.7 times in a shaded area. In addition, an increase in stomata width of abaxial epidermis of cauline leaf was noticed in a sunny area. Comparison of sunny and shaded areas revealed a 1.2-fold decrease in the stomata area in a sunny area. Plants grown in a greenhouse had a significantly smaller number of stomata per 1 mm² of abaxial epidermis and smaller stomata width compared to those in a shaded area; their stomata area was smaller compared to a sunny area. Each group of plants is characterized by its own set of morphological and anatomical features, which can be considered as adaptive potential of species dwelling in an arid environment (Gamaley, 1984). Some authors are of the opinion that a low frequency of stomata in combination with their large size contributes to more effective control of water cycle (Bissing, 1982; Ceulemans, 1978). Others (Buinova, 1988; Esau, 1980) take the opposite view that mesophyll leaves in dry conditions tend to have a greater number of stomata of a smaller size. For a variety of plants, stomata in the fully open form occupy 1-3% of the leaf area, while the diffusion of water vapors from the leaf flows from a leaf with open stomata as fast as from a free surface. Transpiration rate depends on the width of stomatal slits, the difference of water potentials of air inside and outside the leaf and on air turbulence. On a sunny day, the temperature of the leaf can be 10 °C higher than that of the ambient air. Due to this temperature difference, the transpiration increases because the air inside the leaf is saturated with moisture, and because saturation vapor pressure increases with rising temperature. Air turbulence also promotes transpiration because rapid removal of water vapor from adjacent to the leaf air layer increases the diffusion gradient from the leaf to the air. Stomata movements are regulated by the main environmental factors: light, temperature, soil moisture, air humidity and CO_2 concentration in the air. These variables affect the internal factors such as water content and the concentration of abscisic acid in a leaf (Galston *et. al.*, 1983).

There is a direct linear dependence of rosellate leaf green weight on the petiole vascular tissue area on the cross section of its middle part (Fig. 5), with a strong dependence of these parameters observed in a sunny area.

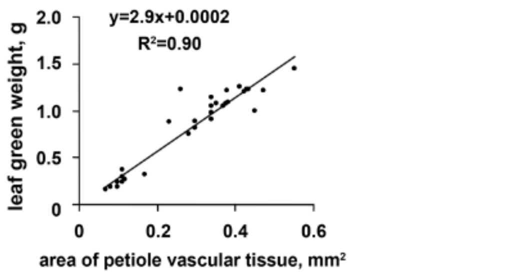

Fig. 5. The dependence of the dry weight of rosellate leaf of *Campanula alliariifolia* Willd on the vascular tissue area of petiole cross section in the steppe zone of Ukraine (greenhouse, sunny area, shaded area)

As the dependence of the leaf dry weight on vascular tissue area was not established, it can be assumed that the larger diameter of petiole vessels results in greater accumulation of water in the leaf cells. Along with other anatomical and physiological processes, which occur in the leaf and contribute to homeostasis maintenance of leaf water balance (such as the rate of photosynthesis, stomata apparatus features, etc), this is adaptation to arid conditions. The leaves of Caucasus species of *C. alliariifolia* in conditions of introduction in the steppe zone have greater frequency and size of stomata. This is evidence of the intense water discharge for leaf cooling. This strategy is possible if the root system is sufficiently developed or if the content of available soil moisture in the root volume is high.

While the petiole cross-section area of the rosellate leaf rises, the collenchyma area increases to provide its strength growth (Fig. 6).

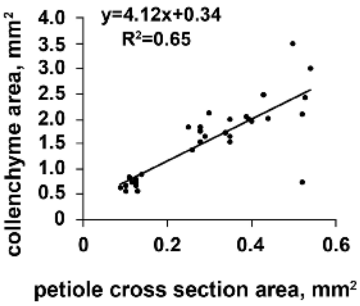

Fig. 6. Dependence of rosellate leaf collenchyma area of *Campanula alliariifolia* Willd. on the cross-section area in the steppe zone of Ukraine (greenhouse, sunny area, shaded area)

Analysis of the dependence of leaf plate green and dry weight on its area showed that the plant *C. alliariifolia* from a natural habitat (city Sochi, forest) with high variation of leaf plate area had a minimal accumulation of green weight per unit area compared to plants in the region of introduction, and the accumulation of dry weight had no significant differences (Fig. 7 a, b). In conditions of introduction region, the index of variation of leaf plate area in a sunny area decreases, the accumulation of green and dry weight per unit leaf area and leaf plate are maximal.

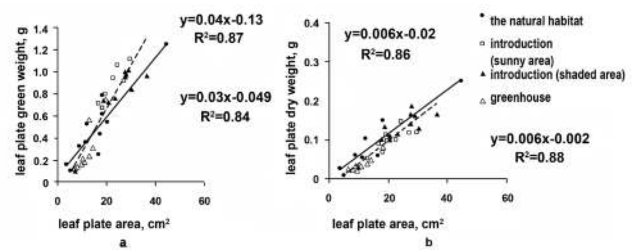

Fig. 7. Dependence of *Campanula alliariifolia* Willd. rosellate leaf weight on the leaf area in different growth conditions: a – green weight; b – dry weight

In shaded locations in the introduction region, the correlation between these parameters declines, the accumulation of leaf plate green weight per unit area is significantly less than in a sunny area. The accumulation of leaf plate dry weight to the area in a shaded area is greater than in a sunny area, but is equal to the natural habitat. The index of leaf plate variation is the same as in a sunny area. In a greenhouse, the leaf plate variation is reduced, the accumulation of green and dry weight per unit leaf area is less compared to the natural habitat, sunny and shaded experimental plots. The ratio of dry weight of the leaf and of the leaf plate to the green weight in a shaded area is significantly greater than in a sunny area The ratio of leaf dry weight to the green weight in a shaded area is significantly less compared to the natural habitat. The character of dependence of accumulation of leaf plate green weight per unit area in the natural habitat and in introduction region changes. However, dependences of these parameters stay the same, which allows the plant to function under changed environmental conditions. The character of dependence of accumulation of leaf dry weight per unit area in the natural habitat and in introduction region is the same. When *C. alliariifolia* rosellate leaf size increases in different habitats the accumulation of its dry weight per area goes up.

The analysis of the correlation dependences of different parameters of *Campanula alliariifolia* in natural place of growth and in the region of introduction revealed that when introduction takes place quantity of links between various morphological, physiological and anatomical parameters increases. This allows us to assume that one of the ways for *Campanula alliariifolia* to adapt to new growth conditions (by increasing intensity of limiting factors) is a simultaneous correlation of changes in several parameters, both morphological and anatomical ones, and increase in link closeness, mainly of allometric parameters with various other indicators. In the natural habitat, the number of stomata per 1 mm² of roselate leaf abaxial surface and its ratio of dry weight to green weight go up while petiole length increases.

In a sunny area in the region of introduction, when columnar mesophyll is rising, the leaf dry weight of the plants and the ratio of the dry leaf plate weight to the green weight increase. Columnar mesophyll activity contributes to the accumulation of plastic materials in the leaf. The ratio of leaf plate green weight to the area declines with the growth in the number of stomata on the abaxial epidermis (transpiration rate increases providing more efficient leaf plate cooling). The ratio of leaf plate dry weight to its green weight increases with rising number of cells per 1 mm² of abaxial leaf surface. With increase in leaf mesophyll thickness, the ratio of leaf plate dry weight to its green weight goes up. The ratio of leaf dry weight to its green weight and the ratio of leaf plate dry weight to the area correlate negatively with the thickness of adaxial epidermis, because a large water amount accumulates in adaxial epidermal cells. There is a direct dependence of the petiole diameter on its length and area, leaf green weight and leaf plate.

In conditions of a shaded area of the introduction region, there is an inverse correlation between the ratio of leaf plate dry weight to green weight and the thickness of adaxial epidermis, a direct correlation between the leaf plate area and the ratio of petiole diameter to its length, and a direct correlation between adaxial epidermis thickness and leaf dry weight. However, these parameters' closeness declines compared to the one in a sunny area. One of the ways to cope with changing growth conditions is the change in the area and weight of photosynthetic organ simultaneously with ensuring of appropriate petiole biomechanics – increase in diameter and decline in petiole length (Fig. 8). In a shaded area, a greater columnar mesophyll thickness and an increase in stomata size are observed. In a greenhouse, when the ratio of petiole diameter to its length increases, the stomata size declines and columnar mesophyll thickness rises. When a rosellate leaf petiole diameter increases, the leaf plate area grows. When columnar mesophyll thickness increases, the leaf plate green weight and dry weight and its area go up. In a greenhouse, direct correlation between the leaf linear dimensions is observed.

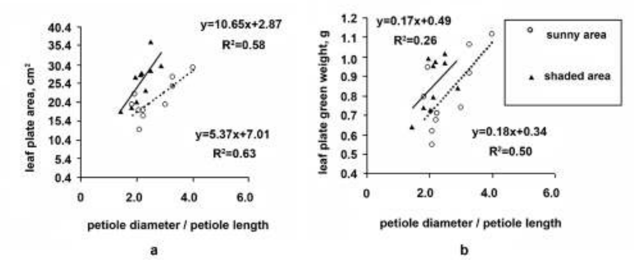

Fig. 8. Dependence of some parameters of *Campanula alliariifolia* Willd. rosellate leaf on the ratio of petiole diameter / petiole length to: a – leaf plate area; b – leaf plate green weight

Analysis of *C. alliariifolia* cauline leaves showed that the introduction of the plant into the steppe zone of Ukraine (a sunny area) reduces the size, index and leaf area, leaf dry weight and simultaneously increases ratio of leaf green and dry weights to the leaf area, leaf thickness, mesophyll, adaxial and abaxial epidermis, the number of cells and stomata on the abaxial epidermis. In a shaded area, the ratio of leaf plate green and dry weights to its area also increases. The thickness of the leaf and of the mesophyll, the number of cells and stomata per 1 mm^2 of adaxial leaf surface also go up.

The leaf index in a sunny area is significantly smaller compared to the natural habitat. In the case of introduction, the cauline leaf dry weight is smaller by 0.93 times, and the petiole length is 4.0 times smaller compared to the natural habitat. In a sunny area of the region of introduction, the ratio of the leaf dry weight to its area is the same for cauline and rosellate leaves of *C. alliariifolia* (Fig. 9).

Conclusion

Thus, the adaptation of *Campanula alliariifolia* to the conditions of introduction region consists of qualitative and quantitative changes of the leaf structure. Qualitative changes regard the formation of columnar mesophyll and stomata on the adaxial side of the leaf. Quantitative ones consist of increase in thickness of leaf and mesophyll and in the stomata frequency and their relative total area, and of greater moisture content per unit area of the leaf.

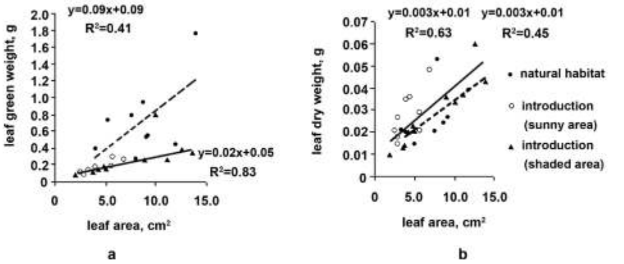

Fig. 9. Dependence of cauline leaf weight of *Campanula alliariifolia* Willd. on the leaf plate area in different growth conditions: a – green weight; b – dry weight

Differences in the direction of changes in the rosellate and cauline leaf structure relate to the leaf area, which increases in rosellate leaves, and to the accumulation of the dry matter per unit area, which increases in cauline leaves. Greater xeromorphy of cauline leaves is linked to the increase in temperature and to the decrease in precipitation amount in the period of their formation. The main ways of *C. alliariifolia* adaptation to new growth conditions are revealed (assuming that intensity of limiting factor activity increases): a) correlation of changes in several parameters, both morphological and anatomical ones, and increased link closeness of the parameters, especially of allometric ones, b) changes in photosynthetic organ weight with simultaneous ensuring of appropriate petiole biomechanics (increase in diameter and petiole length reduction), and c) homeostasis maintenance of leaves of different formations by means of the same physiological and anatomical reconstructions. In the region of introduction, higher insolation leads to the increase in leaf thickness and mesophyll, greater water accumulation per unit leaf area of different formations. It also results in the decrease in the area and in the dry weight of rosellate leaf. Under the conditions of higher insolation, the ratio of leaf dry weight to the area remains constant for leaves of different formations.

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