ANNALI DI BOTANICA
Ann. Bot. (Roma), 2015, 5: 17–29
Journal homepage: http://annalidibotanica.uniroma1.it

FUNCTIONAL MORPHOLOGY OF THE VEGETATIVE ORGANS OF TEN *AQUILEGIA* L. SPECIES

KROKHMAL I.I.

Institute for evolutionary ecology of the Nat. Acad. of Scien. of Ukraine st. acad. Lebedev, 37 Kiev 03143 Ukraine
Telephone: +380954019978; e-mail: dies_irae78@mail.ru

(RECEIVED 11 JULY 2014; RECEIVED IN REVISED FORM 27 NOVEMBER 2014; ACCEPTED 15 JANUARY 2015)

ABSTRACT – Establishment of patterns in the functional morphology of the vegetative organs of plants in new conditions allows to determine traits that are important in adaptation and to predict success of introduction of species in the Ukrainian steppe zone. We analysed the functional morphology of the vegetative organs of 10 *Aquilegia* L. species having applied methods widely used in anatomy, morphology and ecology. The groups of species were compared on the basis of their eco-geographical origin using ANOVA test, as a result of which, we found out diagnostic features of a successful adaptation of some of them. These features are a large volume of the root system, a greater thickness of the hypocotyl, a larger petiole xylem area of the leaf and a higher stomatal index. A diagnostic trait of successful adaptation of species is a smaller value of the ratio of the petiole diameter to its length in comparison with other researched species. Our search for dependencies and determination of their degree revealed that plant biomass, in particular of its above-ground part, and plant petiole parameters (diameter, the area of its cross-section and of its xylem, quantity of the conducting bundles) correlate with the volume of the root system and with the hypocotyl thickness. We analyzed 52 morphological and anatomical attributes of species of the genus *Aquilegia* and 9 climatic factors of their natural habitat. It was detected that ecological and geographical origin of the species affects the anatomical and morphological characteristics of their vegetative organs.

KEYWORDS: TRAITS OF SUCCESSFUL ADAPTATION, CLIMATIC FACTORS, FUNCTIONAL MORPHOLOGY

INTRODUCTION

Aquilegia L. genus (fam. Ranunculaceae) emerged circa 6 million years ago in the mountains of South-Central Siberia. Its diversity is the result of two independent pathways of evolution. For Asian and North American species, it is typical to have sympatric speciation caused by pollinator diversity. For Asian and European species, allopatric speciation is common (Bastida, 2010).

Aquilegia species vary in terms of duration of flowering, ornamental habit, color, flower shape and relative resistance to various growing conditions. There are 60-70 *Aquilegia* species growing in the Northern Hemisphere. 35 of them are introduced into the culture. Currently, in the Donetsk Botanical Garden 20 species, three varieties and two kinds of this genus are collected.

Establishment of patterns in the vegetative organs' functional morphology of *Aquilegia* plants with various ecological and geographical origin in the conditions of introduction allows to determine the most important traits and groups of traits as adaptation to the new conditions. Identification of such a connection allows to predict a successful introduction and retention of species potentially more adapted to the conditions of the Ukrainian steppe.

Adaptation of plants to extreme conditions is associated with significant restructuring of their assimilation system, which is highly sensitive to external influences. The photosynthetic apparatus has a high plasticity resulting in a significant variation of leaf size and its anatomical characteristics (Nicholaevskaya, 1990; Eamus and Prichard, 1998; Zhang et al., 2012). The adaptation process involves the whole plant. Morphological and anatomical modifications linked to this

doi: 10.4462/annbotrm-12458

process change physiological status of the plant. As a result, the plant is able to use more effectively the environmental resources in new extreme conditions of introduction (Menshakova et al., 2008; Sack et al., 2013; Poorter et al., 2014). The activity of various adaptive mechanisms is directed to reduce the effect of the damaging factor to subthreshold values (Crawford, 1989).

There is a large body of studies on the leaf, in particular on the changes in size and density of leaf cells and tissues, the leaf surface and epidermal layers (the stomata complex), internal anatomy (e.g., palisade thickness), and ecophysiological performance (Poorter and Remkes, 1990; Tsialtas et al., 2002; Wright et al., 2004; Franks and Beerling, 2009; Osunkoya et al., 2010; Scheepens et al., 2010; Brodribb et al., 2013). However, the data set on which the studies of adaptive traits are based usually includes features of photosynthetic apparatus of plants, and rarely parameters of other vegetative parts, in particular a root. Hardly ever, conductance of a plant is studied in a leaf-petiole-root complex. Therefore, studying of vegetative organs of plants, establishment of linkages between functional parameters of these organs and their coordinated change in response to the changing environment conditions are of a great interest and make topical the aim of our research.

The goal of this work is to determine the functional morphology characteristics of the vegetative organs of some *Aquilegia* species with different eco-geographical origin, cultivated in the steppe zone of Ukraine, as well as to detect the most important traits and groups of traits as adaptations to new conditions.

Materials and methods

Research materials are plants of 10 *Aquilegia* L. species introduced into the steppe of Ukraine: *A. flabellata* Sieb. et Zucc. var. *pumila*, *A. oxysepala* var. *kansuensis* (Brune) Hand.-Mazz., *A. buergeriana* Sieb. et Zucc., *A. aurea* Janka,

Table 1. Eco-geographical origin of the studied *Aquilegia* L. species, climatic factors of their ranges and of the Ukrainian steppe: Pr – annual precipitation; Ev – annual evaporation; fd – moisture coefficient (Pr/Ev); Pr – Ev – the difference between annual precipitation and evaporation; Σt – temperature sum for the days with temperature above 10°C; twm – temperature of the warmest month, °C; Dp>10°C – the number of days with temperature above 10°C; Dp>5°C – the number of days with temperature above 5°C; Dp>15°C – the number of days with temperature above 15°C.

Eco- geographical origin	**Species**	**Climatic factors**								
		Pr mm	**Ev mm**	**(Pr – Ev) mm**	**∑t °C**	**twm °C**	**Dp >10 °C days**	**Dp >5 °C days**	**Dp >15 °C days**	**fd**
		in the ranges of the species								
Forests of the Far East (group I)	*A. flabellata* var. *pumila*	500-1000	400-800	200-400	1000-3000	16-22	60-180	120-210	60-120	0.8
	A. oxysepala var. *kansuensis*	500-1000	800-1000	-400-200	1000-3500	12-24	60-240	90-240	60-150	1.0-1.6
	A. buergeriana	1000-2000	800-1000	200-2200	3000-5000	22-26	180-240	180-300	120-150	0.5-0.8
Mountain forests of the Balkans (group II)	*A. aurea*	500-1000	800-1250	-400-(-800)	3000-5000	20.0	180-300	120-240	120-210	1.25-1.6
	A. nigricans	500-1000	800-1250	-400-(-800)	3000-5000	20.0	180-300	120-240	120-210	1.25-1.6
Highland forests of North America (group III)	*A. canadensis*	500-1000	400-1500	-200-200	2000-5000	18-26	120-270	120-300	60-240	0.8-1.5
	A. skinneri	1000-3000	1250-1500	400-2000	6000-9500	18-28	365	365	365	0.5-1.25
Sub-alpine and alpine meadows of Europe (group IV)	*A. atrata*	1000-2000	800-1000	200-1200	1000-2500	4-20	60-180	60-240	60-180	0.5-0.8
	A. alpina	1000-2000	800-1000	200-1200	1000-2500	4-20	60-180	60-240	60-180	0.5-0.8
	A. einseliana	1000-2000	800-1000	200-1200	1000-2500	4-16	60-120	60-180	60-120	0.5-0.8
		in the Ukrainian steppe								
		375	1625	-300	3500	20	150	210	150	0.23

A. nigricans Baumg., *A. canadensis* L., *A. skinneri* Hook., *A. atrata* W.D.J. Koch, *A. alpina* L. and *A. einseliana* F.W. Schultz.

The seeds from Delectus Seminum exchange programme were used as an initial mobilization material of *Aquilegia* species. Eco-coenotic character of the species and agro-climatic indicators of their ranges (Agro-climatic world atlas, 1972) are shown in Table 1.

Leaves, roots and above-ground parts of plants were harvested. 10 plants of each species (10 leaves, roots and above-ground parts of plants per species) were weighed and scanned. The leaf areas and perimeters were calculated using AxioVision software. Thereafter, the scanned leaves, roots and above-ground parts of plants were dried to constant mass at 60°C. They were then weighed to estimate $Wapp/S_{lp}$ (the ratio of the weight of a fresh above-ground plant part to the leaf surface area), $Wapp_2/S_{lp}$ (the ratio of the weight of a dried above-ground plant part to the leaf surface area), Wr/Wp (the ratio of the weight of a fresh root to the plant), Wr_2/Wp_2 (the ratio of the weight of a dry root to the plant), Wr/Wapp (the ratio of the weight of a fresh root to an above-ground plant part), $Wr_2/Wapp_2$ (the ratio of the weight of a dry root to an above-ground plant part), Wp/Vr (the ratio of the weight of a fresh plant to the root volume), Wapp/Vr (the ratio of the weight of an above-ground part to the root volume), M/S (the ratio of the weight of a fresh leaf plate to its area), M_2/S (the ratio of the weight of a dry leaf plate to its area), m/S (the ratio of the weight of a fresh leaf to its area), m_2/S (the ratio of the weight of a dry leaf to its area). These data were also used to calculate the perimeter (Pl) / area (S) ratio for each leaf [i.e., the dissection of the leaf (Dis)] (Sack et al., 2003). The volume of the root system was measured by an immersion method. For each plant, we determined the height (hp) and the diameter (Dp); the number of leaves (nl), the leaf area (S_{lp}); the diameter (dr), the length (Lr) and the volume of the root system (Vr); the number of adventitious roots (Nar) and their length (Lar); the size of the hypocotyl (Lh, th), and the number of regeneration buds (Nrk).

We examined 10 leaves of plants of each species. Temporary anatomical preparations of a leaf were used. The cut of the leaf plate and of the petiole was done in their middle part. The thickness of the leaf, epidermis and mesophyll were measured at equal distance between the edge of the leaf and its main vein. In this work, we used a microscope Zeizz Primo Star and AxioVision Software for measurement purposes. Microreplication technique (Klein, 1974) was applied to arrange preparations of leaf epidermis. The ratios of the tissue layers to the total leaf thickness and the ratio of palisade to spongy mesophyll were calculated (Boyne, 2011; Boyne et al., 2013). The counts of stomata and pavement cells were used to calculate the stomatal-epidermal index (SI) in % equal to Ns * 100/(EC + Ns) (where Ns is the number of stomata and EC is the number of epidermal cells per 1 mm^2 of the leaf surface). This provides a measure of the number of stomata that have developed within a given area without the confounding effect of pavement cell expansion (Gupta, 1961). We also determined the petiole diameter (dp) and length (Lp); the thickness of the epidermis (tep), chlorenchyma (thl) and sclerenchyma (tsc) of the petiole; the number of conducting bundles in the petiole (Ncb); the petiole cross-sectional area (Sp); the parenchyma (Sp_2), sclerenchyma (Ssc), conducting bundles (Scb) and xylem areas (Sxl). We calculated the coefficient dp/Lp – the ratio of the petiole diameter to its length, which is an adaptive trait (Krokhmal, 2013). Comparison of the groups of species by their eco-geographical origin was carried out using the statistical test ANOVA.

Results

Anatomical and morphological traits of the vegetative organs

Leaf of *Aquilegia* species is ternate and dorsiventral. A typical characteristic of the leaf surface is the lotus effect which occurs due to the peculiarities of the microstructure of the leaf surface and its high hydrophobicity. The lotus effect refers to water drops rolling off the leaf and cleaning its surface. It increases efficiency of photosynthesis, plant protection against microorganisms and fungi. The type of stomata is anomocytic. Epidermal cell walls are flexuous. Below petiole epidermis there are 3-5 layers of mesophyll and several layers of sclerenchyma. The thickness of sclerenchyma increases at the base of the conducting bundles. Bundles are arranged in a circle, large alternating with small. The petiole pith is composed of parenchyma. Sometimes in the middle of the petiole aeriferous cavity is formed (Fig. 1).

Petiole and leaf surface of some examined species (*A. oxysepala* var. *kansuensis, A. canadensis, A. skinneri, A. alpina, A. nigricans* and *A. atrata*) is covered with trichomes. A higher leaf hair density contributes to a low leaf boundary layer, low transpiration, and protection from excessive radiation (Roy et al., 1999).

In the conditions of the Ukrainian steppe, 2 layers of columnar mesophyll are formed in *A. einseliana*; 2-3 layers in *A. oxysepala* var. *kansuensis, A. canadensis, A. skinneri* and *A. alpina*; 3 layers in *A. flabellata* var. *pumila* and *A. buergeriana*; 3-4 layers in *A. nigricans, A. atrata* and *A. aurea* leaves. In the conditions of the introduction region, the life-form of the majority of the examined species is semi-rosette fibrous root hemicryptophyte. The life-form of

A. flabellata var. *pumila* is fibrous-taproot hemicryptophyte. The species are not capable of natural vegetative reproduction. The root system of the examined *Aquilegia* species is represented by many-headed caudex formed from lignified base shoots and a thickened hypocotyl. On the caudex, adventitious roots are formed. They branch out to the 4th order. Each caudex head consists of a caudex generative shoot and several vegetative shoots of the second order. The caudex has contractile properties, allowing the plant to move deep into the soil and protect its regeneration buds upon the occurrence of adverse conditions. Leaf rosette of *Aquilegia* species also protects the buds from extreme conditions.

Trait mean and differences between groups of species of different eco-geographical origin

Adaptation can be promoted by higher values of functional traits, higher phenotypic plasticity and phenotypic integration. It can also be a consequence of positive relative influence of these three functionalities on fitness. Many leaf anatomical and root morphological traits differed between groups of *Aquilegia* species of different eco-geographical origin, which led to different adaptation to the conditions of introduction region. In the Ukrainian steppe, the Balkan mountain forest species (group II) differ by a higher degree of adaptation. This group of *Aquilegia* species is different from others by higher values of the root diameter ($p<0.042$) and volume ($p<0.05$), the thickness hypocotyl ($p<0.05$), the petiole cross-sectional area ($p<0.05$), the petiole sclerenchyma ($p<0.04$) and pare nchyma ($p<0.05$), the number of stomata per 1 mm^2 of a lower epidermis ($p<0.05$) and the stomatal index (SI) ($p<0.029$). Compared with other examined groups, group II of the species has higher ratios Wp/Vr and Wapp/Vr, and lower ratios Wr/Wp and W_2r/W_2p ($p<0.05$) (Tables 2-5).

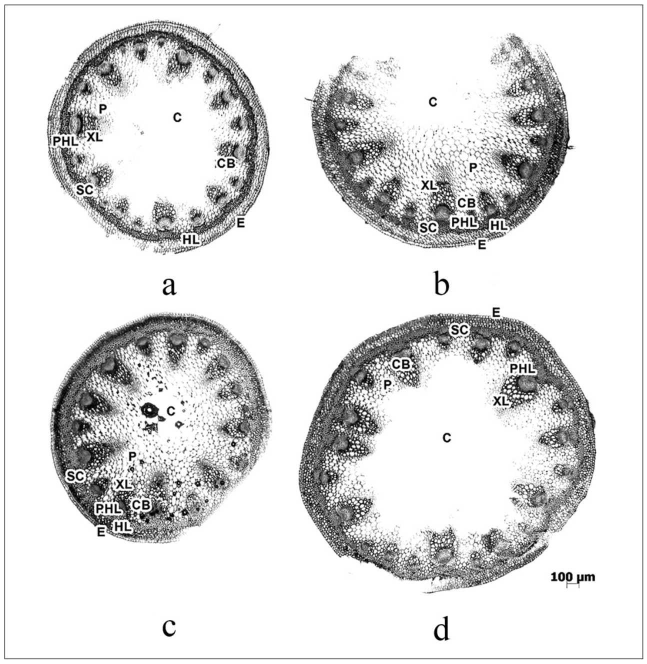

Fig. 1. Anatomical structure of the leaf petiole of *Aquilegia* species of a different eco-geographical origin: a – *A. oxysepala* var. *kansuensis* (group I), b – *A. aurea* (group II), c – *A. canadensis* (group III), d – *A. alpina* (group IV); E – epidermis, SC – sclerenchyma, HL – chlorenchyma, P – parenchyma, C – cavity, XL – xylem, PHL – phloem, CB – conducting bundle.

Table 2. Morphological traits of the root system and coefficients (ratios of parameters of above-ground and under-ground parts of plants) of *Aquilegia* L. species grouped by their eco-geographical origin: dr – diameter, Lr – length, Vr – volume of the root system, th – thickness of the hypocotyl, Nar – the number of adventitious roots, Wp/Vr – the ratio of the weight of a fresh plant to the root volume, Wapp/Vr – the ratio of the weight of an above-ground part to the root volume, Wr/Wp – the ratio of the weight of a fresh root to the plant, Wr_2/Wp_2 – the ratio of the weight of a dry root to the plant; the table shows average value of the parameters ± standard deviation.

Group	dr cm	Lr cm	Vr cm^3	th cm	Nar p.	Wp/Vr	Wapp/Vr	Wr/Wp	Wr_2/Wp_2
I	8.27 ± 3.74	19.04 ± 4.23	11.84 ± 7.04	1.33 ± 0.37	7.63 ± 1.87	2.56 ± 1.23	1.41 ± 0.88	0.47 ± 0.12	0.18 ± 0.08
II	12.26 ± 4.74	18.96 ± 3.40	15.88 ± 8.92	1.49 ± 0.47	8.87 ± 4.03	2.92 ± 0.83	1.67 ± 0.52	0.39 ± 0.04	0.14 ± 0.09
III	9.11 ± 4.69	18.59 ± 4.08	10.93 ± 8.00	1.45 ± 0.47	7.40 ± 3.80	2.72 ± 0.87	1.44 ± 1.06	0.41 ± 0.15	0.26 ± 0.21
IV	8.20 ± 4.10	20.12 ± 4.14	12.69 ± 7.01	1.32 ± 0.44	9.07 ± 4.70	2.63 ± 1.30	1.16 ± 1.47	0.44 ± 0.10	0.13 ± 0.07

Table 3. Morphological traits of the leaf of the *Aquilegia* L. species grouped by their eco-geographical origin: Pl – perimeter, S – area, S_{lp} – the surface area of the leaf, m – the weight of a fresh leaf, M – the weight of a fresh leaf plate, m_2 – the weight of a dry leaf, M_2 – the weight of a dry leaf plate, m_2/m – the ratio of the weight of a dry leaf to a fresh one, Dis – the dissection of the leaf, m_2/S – the ratio of the weight of a dry leaf to its area; the table shows the average value of the parameters ± standard deviation.

Group	Pl cm	S cm^2	S_{lp} cm^2	m g	M g	m_2 g	M_2 g	m_2/m	Dis	m_2/S
I	61.6 ± 31.58	40.6 ± 20.58	579.1 ± 443.07	1.84 ± 0.797	1.42 ± 0.644	0.36 ± 0.211	0.25 ± 0.172	0.17 ± 0.055	1.7 ± 0.78	0.0058 ± 0.0022
II	86.6 ± 24.29	59.4 ± 24.38	1104.76 ± 773.97	2.55 ± 0.364	1.64 ± 0.323	0.50 ± 0.079	0.25 ± 0.040	0.16 ± 0.004	1.5 ± 0.37	0.0043 ± 0.0002
III	95.9 ± 18.65	52.6 ± 14.41	829.01 ± 582.87	2.47 ± 0.679	1.73 ± 0.245	0.58 ± 0.118	0.38 ± 0.029	0.22 ± 0.014	1.9 ± 0.38	0.0073 ± 0.0003
IV	90.9 ± 26.38	73.2 ± 25.79	1111.18 ± 615.69	2.82 ± 0.558	2.15 ± 0.366	0.45 ± 0.094	0.28 ± 0.041	0.13 ± 0.009	1.3 ± 0.24	0.0039 ± 0.0004

Table 4. Anatomical traits of the leaf of the *Aquilegia* L. species grouped by their eco-geographical origin: tpm – the thickness of the columnar mesophyll, tsm – the thickness of the spongy mesophyll, tue – the thickness of the upper epidermis, tie – the thickness of the lower epidermis, fp – the palisade index, Ns – the number of stomata per 1 mm^2 of the leaf surface, Ls – stomata length, ts – stomata thickness, SI – the stomatal index in %; the table shows the average value of the parameters ± standard deviation.

Group	tpm mcm	tsm mcm	tue mcm	tie mcm	fp	Ns p.	Ls mcm	ts mcm	SI
I	98.8 ± 12.57	98.8 ± 23.13	33.6 ± 6.78	28.9 ± 3.64	0.5 ± 0.05	178.6 ± 43.37	31.0 ± 5.04	24.9 ± 2.32	32.0 ± 20.00
II	99.6 ± 23.75	99.6 ± 44.40	29.9 ± 5.75	26.8 ± 4.56	0.5 ± 0.07	209.0 ± 84.35	30.8 ± 3.58	24.9 ± 1.09	67.2 ± 5.82
III	89.4 ± 17.44	89.4 ± 16.34	30.7 ± 5.40	29.5 ± 8.21	0.6 ± 0.04	182.2 ± 44.69	30.8 ± 4.41	23.5 ± 2.98	61.2 ± 11.87
IV	106.7 ± 25.53	106.7 ± 23.38	39.0 ± 16.03	29.2 ± 6.51	0.5 ± 0.09	201.6 ± 47.70	29.2 ± 4.32	24.1 ± 3.79	65.3 ± 6.41

Sub-alpine and alpine species of Europe (group IV) have a deep root system ($p<0.05$), a large number of adventitious roots ($p<0.05$), and a greater leaf surface area ($p<0.005$). This group of species differs by a larger thickness of columnar mesophyll and epidermis, a higher value of the palisade index (fp) ($p<0.05$) (Fig. 2), a higher stomata density of a lower epidermis ($p<0.05$) and of the conducting bundles in the leaf petiole ($p<0.0005$). It is well known that a large number of small stomata maximizes gaseous CO_2 diffusion into leaves for photosynthesis without excessive water loss (Holland and Richardson, 2009; Brodribb et al., 2013). Group IV of *Aquilegia* species is also characterized by higher values of the leaf and the leaf plate weight ($p<0.003$, $p<0.05$; $p<0.002$, $p<0.004$ – respectively, for fresh and dry ones), smaller values of the dissection of the leaf and of the ratios Wr_2/Wp_2 ($p<0.05$) and m_2/m ($p<0.009$). Similar to the Balkan mountain forest species (group II), species of group IV have a high value of the stomatal index (SI) ($p<0.03$).

Highland forest species of North America (group III) adapt to the Ukrainian steppe due to higher values of the palisade

Table 5. Anatomical and morphological traits of the leaf petiole of *Aquilegia* L. species grouped by their eco-geographical origin: dp – the petiole diameter, Sp – the cross-sectional petiole area, Ssc – the sclerenchyma area of petiole, Sp_2 – the parenchyma area of petiole, Ncb – the number of conducting bundles in petiole, Scb – the area of conducting bundles of petiole, Sxl – the xylem area of petiole, tep – the thickness of petiole epidermis, tsc – the thickness of petiole sclerenchyma; the table shows average value of the parameters ± standard deviation.

Group	dp mm	Sp mm^2	Ssc mm^2	Sp_2 mm^2	Ncb p.	Scb mm^2	Sxl mm^2	tep mcm	tsc mcm
I	2.04 ± 0.225	3.12 ± 0.606	0.35 ± 0.068	1.65 ± 0.362	18.9 ± 1.83	0.54 ± 0.130	0.22 ± 0.155	23.18 ± 4.02	57.70 ± 14.28
II	2.38 ± 0.443	4.32 ± 1.564	0.49 ± 0.131	2.29 ± 1.163	18.5 ± 3.60	0.81 ± 0.168	0.40 ± 0.098	24.22 ± 4.11	70.25 ± 19.60
III	2.18 ± 0.455	3.62 ± 1.482	0.48 ± 0.178	1.53 ± 0.973	17.0 ± 1.63	0.95 ± 0.833	0.45 ± 0.313	26.89 ± 9.66	76.19 ± 13.22
IV	2.25 ± 0.324	3.89 ± 1.099	0.43 ± 0.130	2.15 ± 0.736	21.4 ± 2.50	0.71 ± 0.18	0.36 ± 0.139	22.01 ± 5.57	58.35 ± 11.22

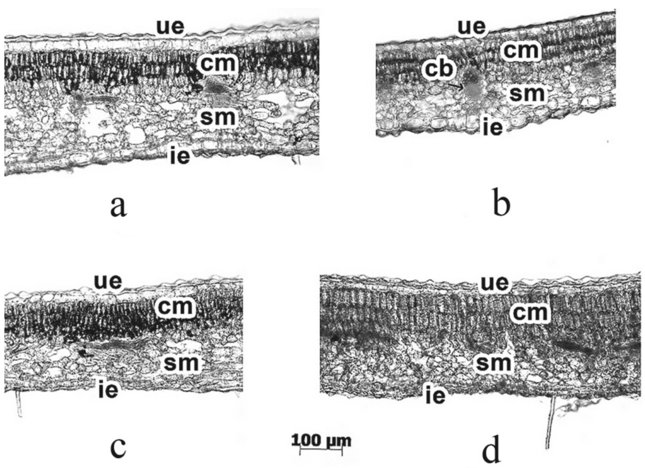

Fig. 2. Anatomical structure of the leaf of *Aquilegia* species of different eco-geographical origin: a – *A. oxysepala* var. *kansuensis* (group I), b – *A. aurea* (group II), c – *A. canadensis* (group III), d – *A. alpina* (group IV); ue – upper epidermis; ie – lower epidermis; sm – spongy mesophyll; cm – columnar mesophyll, cb – conducting bundle.

index (fp) ($p<0.05$) and of the dissection of the leaves ($p<0.04$), as a result of which the leaves are able to cool down quickly and effectively at high summer temperatures. Group III species are characterized by higher values of the area of conducting bundles (Scb) ($p<0.004$) and of the petiole xylem (Sxl) ($p<0.03$). This group differs by small values of the root system volume (Vr) ($p<0.05$), thickness of spongy mesophyll ($p<0.05$), by higher values of thickness of petiole epidermis ($p<0.05$) and of petiole sclerenchyma ($p<0.04$), higher ratios m_2/S ($p<0.005$) and m_2/m ($p<0.009$). This group also differs by the percentage of tissues in leaf petioles: conducting tissues (their area is 1.4-1.5 times larger in comparison with other examined groups of species) and mechanical tissues (the area is 1.3 times larger) are more developed.

Group I of *Aquilegia* species of lowland forests of the Far East differs by maladaptive traits in the conditions of the introduction region compared to other examined species. Group I of species are characterized by lower values of perimeter and area of the leaf ($p<0.04$), of accumulation of water per unit of leaf surface area ($Wapp/S_{ls}$) ($p<0.002$), of petiole length ($p<0.02$); by less developed mechanical ($p<0.04$) and conducting tissues of the leaf petiole ($p<0.004$); by large stomata and their smaller quantity per 1 mm^2 of a lower epidermis ($p<0.05$).

A common adaptive trait of the mountain forest species of the Balkans (group II) and of the sub-alpine and alpine species of Europe (group IV) is a higher stomatal index (SI). Common adaptive traits of the sub-alpine and alpine species of Europe (group IV) and of the highland forest species of North America (group III) are thickening of the lower epidermis, which accumulates water, and a higher index of leaf palisade.

Relationship between morpho-physiological and anatomical traits of vegetative organs

Adaptive plasticity in functional traits is likely to assist rapid adaptation to a changed environment (Nicotra et al., 2010; Gianoli and Valladares, 2012). We detected a significant correlation of a plant total biomass with morphological and

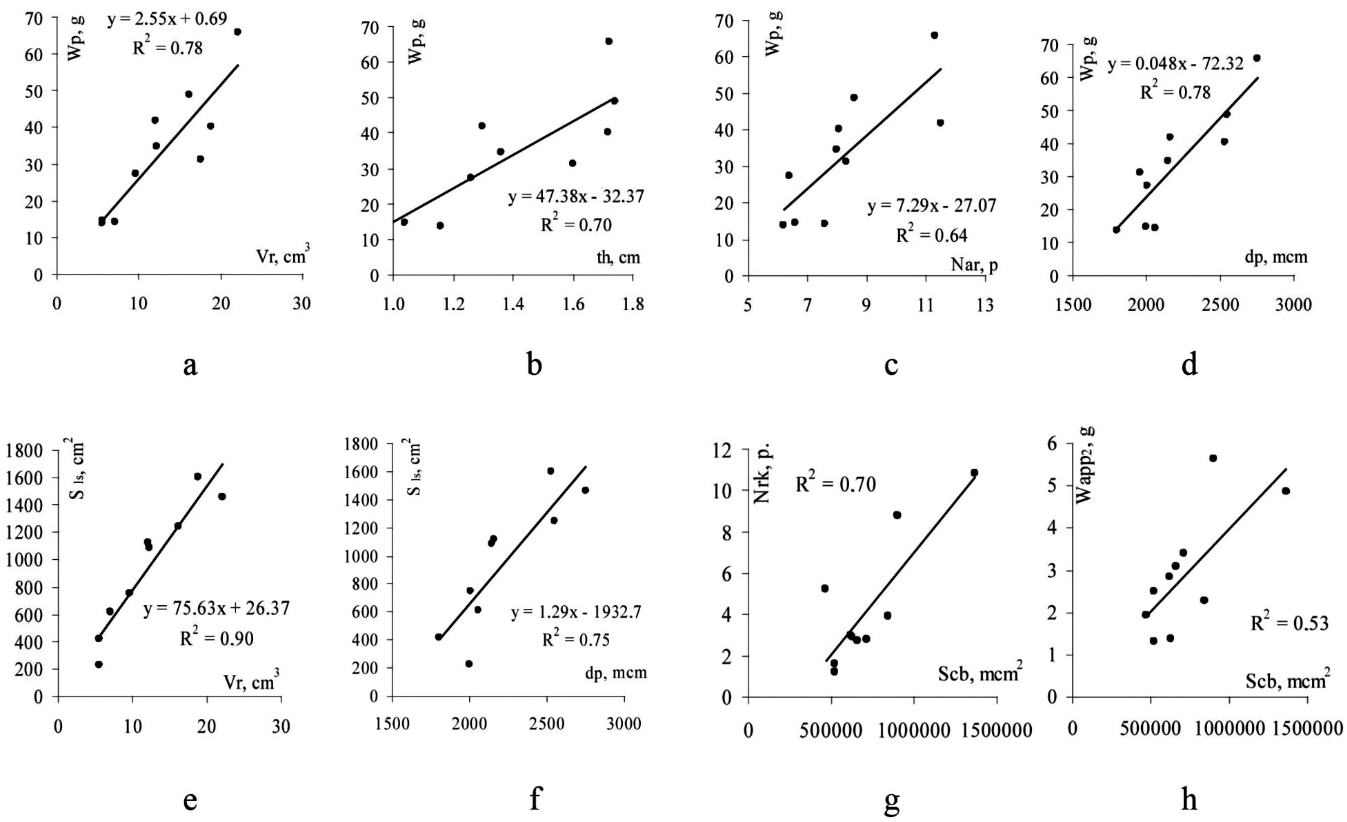

Fig. 3. Relationships between parameters of the above-ground part of plants of the *Aquilegia* species and parameters of the under-ground part and of the leaf petiole: between the weight of a fresh plant (Wp) and the volume of the root system (Vr) (a), the hypocotyl thickness (th) (b), the number of adventitious roots (Nar) (c) and the petiole diameter (dp) (d); between leaf surface area (S_{ls}) and the volume (Vr) of the root system (e), the petiole diameter (dp) (f); the area of the conducting bundles of the leaf petiole (Scb) and the number of regeneration buds (Nrk) (g), the weight of a dry plant above-ground part ($Wapp_2$) (h); every point on the graphs is the average value of the parameter of plants of one species.

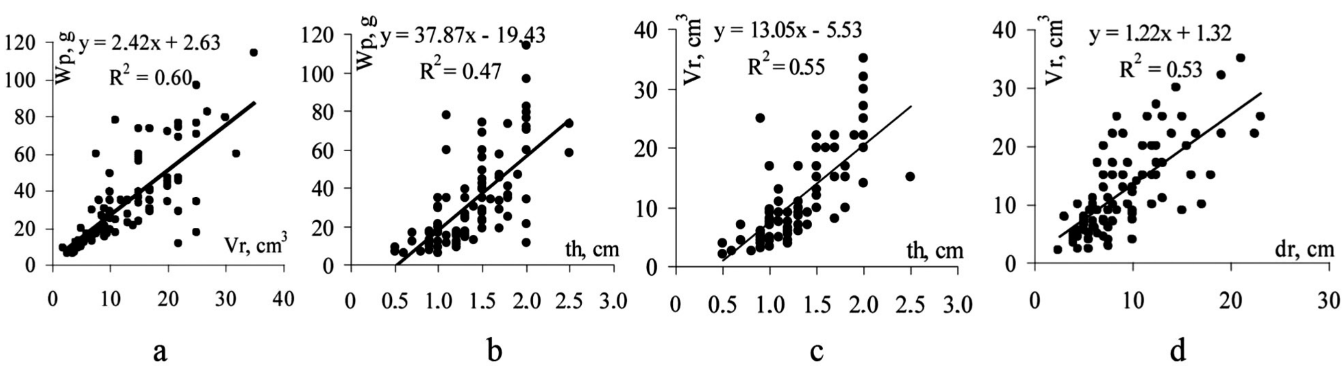

Fig. 4. Relationships between the weight of a fresh plant (Wp) of *Aquilegia* species and the root volume (Vr) (a), the hypocotyl thickness (th) (b), between the root volume (Vr) and the hypocotyl thickness (th) (c), the diameter of the root system (dr) (d); based on the common pool of data.

anatomical traits of its above-ground (>40 traits) and under-ground (8 traits) parts. The weight of a fresh plant (Wp) of *Aquilegia* species increases with enlarged volume (Vr) and diameter (dr) of the root system (R^2=0.77), hypocotyl thickness (th) and a higher number of adventitious roots (Nar) (Fig. 3a-c), as well as larger petiole diameter (dp) (Fig. 3d). The weight of an above-ground part of a fresh (Wapp) and of a dry plant ($Wapp_2$) also increases with enlarged volume (Vr) and diameter (dr) of the root system, and petiole diameter (dp).

Leaf surface area (S_{ls}) of *Aquilegia* species increases with the increase of the root volume (Vr) and petiole diameter (dp) (Fig. 3e-f), the number of adventitious roots (0.62) and of their length (0.66), the stomatal index (IS) (0.65) and the petiole size (0.84).

Analysis of the common pool of data revealed significant correlations between the weight of a fresh plant (Wp) and its above-ground part (Wapp), the petiole diameter (dp), its cross-sectional area (Sp), parenchyma area (Sp_2) and xylem area (Sxl), and the number of conducting bundles in the petiole (Ncb). The weight of a fresh plant (Wp) is also linked with the root system volume (Vr) and the thickness of the hypocotyl (th) (Fig. 4a-b). The root system volume (Vr) is correlated with the thickness of the hypocotyl (th) and the root system diameter (dr) (Fig. 4c-d).

For the common pool of data, a significant positive relationship between the number of regeneration buds (Nrk) of *Aquilegia* species and the conducting bundles area of the leaf petiole (Scb) (0.80) is also typical. The xylem area of the petiole (Sxl) linked with the hypocotyl thickness (th) (0.75).

We established a significant positive correlation between the biomass of above-ground and under-ground parts of plant and the morphological and anatomical traits of the leaf of *Aquilegia* species (according to the average values of the parameters of plants of one species):

1) correlation of the leaf thickness (tl) (correlation coefficient 0.67), columnar mesophyll (tpm) (0.75) and upper epidermis (tue) (0.69) with the number of adventitious roots (Nar);

2) correlation of the conducting bundles area of the petiole (Scb) with the hypocotyl thickness (0.66), the number of regeneration buds (Nrk) (0.84) (Fig. 3g) and the weight of a dry above-ground part of the plant (0.73) (Fig. 3h);

3) correlation of the petiole cross-sectional area (Sp) and of its sclerenchyma area (Ssc) with the root diameter (dr) (0.82 and 0.78, respectively), the root volume (Vr) (0.82 and 0.73), the hypocotyl thickness (th) (0.77 and 0.80), and the number of regeneration buds (Nrk) (0.77 and 0.84);

4) correlation of the leaf area (S) and of the total surface of the leaf (S_{ls}) with adventitious roots' parameters: their number (Nar) (0.70) and length (Lar) (0.66).

The number of leaves (nl) of the plant is linked with the root volume (Vr) (0.64) and the petiole diameter (dp) (0.88). The increasing leaf dissection (Dis) of *Aquilegia* species was accompanied by a decline in the number of conducting bundles (-0.86) and an increase in the columnar mesophyll thickness (0.64).

The height, diameter and weight of the plant, as well as the length, diameter and volume of the root system and the number of adventitious roots, correlated with the petiole size. These parameters are closely related to the area of conducting bundles of the petiole (0.95).

The ratio of the weight of a fresh above-ground plant part to the leaf surface area ($Wapp/S_{ls}$) is negatively correlated with the stomatal index (SI) (-0.87). This is explained by the fact that a greater number of stomata leads to a faster evaporation of water from the leaf surface. The weight of a fresh leaf is linked with the root length (0.70), the number (0.70) and length of adventitious roots (0.72), the petiole size (0.77), the areas of conducting bundles (0.73) and of petiole xylem (0.75), i.e. development of a vigorous root system leads to accumulation of more water in the leaf. The weight of a dry leaf (m_2) is correlated with the petiole length (0.76), the areas of conducting bundles of the leaf petiole (0.73), of petiole xylem (0.73) and of sclerenchyma (0.67), and with the stomatal index (SI) (0.71).

We determined the adaptive trait of *Aquilegia* species developed in response to the conditions of the Ukrainian steppe. This is the ratio of the petiole diameter to its length (dp/Lp). For plants of *Aquilegia* species, increased dp/Lp was accompanied by decreased plant height (hp) and diameter (d), root diameter (dr), hypocotyl thickness (th), areas of conducting bundles (Scb) and petiole xylem (Sxl), stomatal

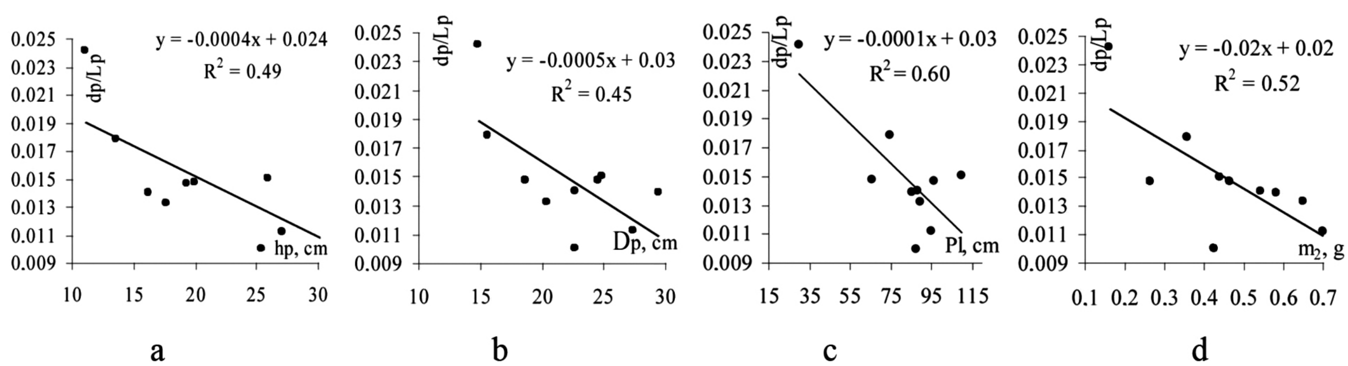

Fig. 5. Relationships between the ratio of the petiole diameter to its length (dp/Lp) of *Aquilegia* species and the plant height (hp) (a), the plant diameter (Dp) (b), the leaf perimeter (Pl) (c) and the weight of a dry leaf (m_2) (d); every point on the graphs is the average value of the parameter of plants of one species.

index (SI), leaf perimeter (Pl) and weight of a dry leaf (m_2) (Fig. 5).

Negative relationships exist between the ratio of petiole diameter to its length (dp/Lp) of *Aquilegia* species and the area of petiole conducting bundles, the area of the large conducting bundle (hence, the hydraulic conductivity of the petiole), the stomatal index (IS), the leaf perimeter (P), the plant height and the weight of a dry leaf. There is a positive relationship between dp/Lp and the weight of a freash leaf (Fig. 6). Consequently, low values of the ratio dp/Lp indicate a higher adaptation of the species to new growth conditions

IV. Relationship between morpho-anatomical traits of the vegetative organs and climatic factors

We detected a link between anatomical and morphological traits of the vegetative organs of *Aquilegia* species and climatic factors of their natural habitat. The petiole length of the leaf increases with increasing sum of temperatures for a time period with the temperature of above 10°C (Σt above 10°C) (0.63) and number of days with the temperature above 10°C (Dp>10°C) (0.66) and 15°C (Dp>15°C) (0.68). Dissection of the leaf (Dis) increases in the species from

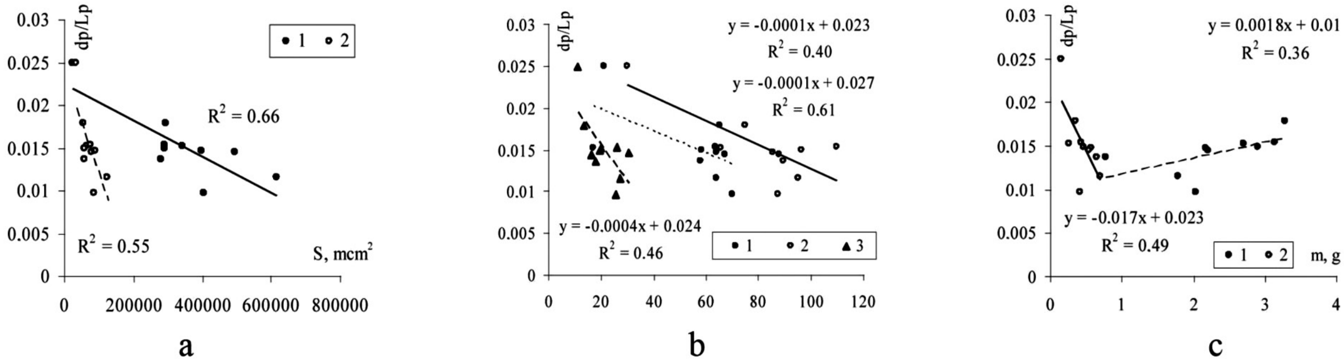

Fig. 6. Relationships between the ratio of the leaf petiole diameter to its length (dp/Lp) of *Aquilegia* species and: a – the xylem area of the petiole (1), the area of the large conducting bundle (2); b – the stomatal index (1), the leaf perimeter (2), the plant height (3); c – the weight of a fresh (1) and of a dry leaf (2); every point on the graphs is the average value of the parameter of plants of one species.

regions with a high temperature of the warmest month (twm) (0.85) and a time period with the temperature above 5°C (Dp>5°C) (0.67).

The ratios of the weight of a fresh and a dry leaf to its area (m/S and m_2/S, respectively) increase with a rise in annual evaporation, sum of temperatures above 10°C (Σt above 10°C), and time periods of temperatures above 10°C (Dp>10°C), 5°C (Dp>5°C) and 15°C (Dp>15°C) in the natural habitat of species.

The *Aquilegia* species from warmer regions with a higher evaporation have larger areas of conducting bundles of petiole (Scb) and petiole xylem (Sxl) (0.70), thickness of epidermis (tep) and of sclerenchyma of petiole (tsc) (0.60) compared with other species.

In the Ukrainian steppe, the *Aquilegia* species from the regions with lower annual precipitation (Pr) are characterized by the development of spongy mesophyll (tsm) accumulating water, large stomata and by higher values of ratios Wapp/Vr and $Wapp_2/S_{lp}$. The species from the regions with a large annual precipitation (Pr) have higher values of the thickness of lower epidermis and palisade index (fp).

We observed that plants of *Aquilegia* species from more humid habitats (species of the Far Eastern forest and North American mountain forests) have a tendency to form smaller diameter and volume of the root system, fewer rosettes and leaves. They differ by smaller weight of a fresh plant and of a dry above-ground plant part, lower values of ratios Wp/Vr and Wapp/Vr, and thickness of the lower epidermis.

In the Ukrainian steppe, the plants of *Aquilegia* species (European alpine and sub-alpine, Far Eastern forest species) from colder habitats develop a long root system (Lr), increased thickness of upper epidermis (tue) and of columnar mesophyll (tpm) of leaves and number of conducting bundles in the leaf petiole (Ncb). They have a decreased thickness of epidermis and sclerenchyma of petiole, dissection of leaf and amount of regeneration buds, and value of the ratio of the weight of a dry to a fresh leaf (m_2/m).

The plants of *Aquilegia* species (European alpine and sub-alpine ones) from the regions with a high solar radiation in June differ by a long root system and a thicker leaf epidermis.

Discussion

Adaptive traits: relationships and integration

Allometric scaling models describing size-dependent biological relationships are important for understanding the adaptive responses of plants to environmental changes (Niklas, 1994; Hui et al., 2012). Morphological changes of a leaf are a functional response of plants to the environmental factors (Zhongqiang et al., 2009; Scheepens et al., 2010).

A formation of columnar mesophyll, an increase in palisade tissue layers and in their height – all these are xeromorphic characteristics of mesophytes in arid growth conditions. It is well known that growth in arid conditions and a high insolation leads to an increase in the leaf plate thickness and assimilative tissue (Vasilevskaya, 1954; Dyachenko, 1978; Osunkoya et al., 2014). An increase in the number of layers and thickness of palisade mesophyll has adaptive character, because the main photosynthetic activity of a leaf is connected precisely with palisade tissue. This is due to the fact that it takes much longer for new chloroplasts to grow in palisade tissue (Menshakova et al., 2008). Epidermal cells of some species of the genus *Campanula* perform the function of water storage (Gyorgy, 2009), therefore, an increase in epidermis thickness is an adaptive response to the lack of humidity in the habitat.

The adaptive traits of sub-alpine and alpine *Aquilegia* species of Europe are an increase in thickness of columnar mesophyll, palisade index (fp) and number of small stomata per 1 mm^2 of the lower leaf surface compared with other species. The adaptive trait of Balkan mountain forest species and sub-alpine and alpine species of Europe is a high value of the stomatal index (SI).

It is known that, taken together, the higher plasticity and phenotypic integration of leaf stomatal complex with many aspects of carbon economy may permit invasive species to occupy broader ecological and environmental niches than that possible for native species (Funk, 2013; Osunkoya et al., 2010). Therefore, we assume that these parameters determine the success of the adaptation of the species to new growth conditions.

Some authors (Esau, 1980; Buinova, 1988; Holland and Richardson, 2009; Brodribb et al., 2013) take the view that in dry conditions leaves of mesophytes tend to have more stomata of smaller size, which allows the plants to adapt to new habitat conditions. According to other authors, combination of a low number of stomata with their large size contributes to a more effective control of the water cycle (Ceulemans, 1978; Bissing, 1982). The species of lowland forests of the Far East differ by large stomata and their smaller density. Consequently, this trait is useful for these species as adaptation to the new region conditions.

Large leaf dissection allows the leaf to cool down in a dry growing environment (Vogel, 2009). An intensive evaporation of a hot humid air can severely damage an entire leaf. In the case of a dissected leaf plate, convection currents pass between leaf segments, which allows the leaf to cool down faster and more evenly. The adaptive trait of the species of North America is a greater dissection of the leaf. Mechanical properties of the leaf of this species depend on the sclerenchyma development rather than on the accumulation of water in the leaves. The leaf petiole of the highland forest species of North America has more developed conducting tissues compared with other studied species. Compared to the climate of the natural habitat of the North American highland species, the climate of the Ukrainian steppe is drier and colder, and solar radiation in June is higher. This leads to adaptive functional morphological reorganization of the leaf, which results in a strong development of conducting and mechanical tissues.

Higher values of adaptive traits (e.g., photosynthetic capacity, biomass accumulation) and greater plasticity and coordination of such traits (Osunkoya et al., 2014) contribute to adaptation of plant species to new habitat conditions. However, the existing data on such traits rarely includes relationships between a morpho-anatomical structure of a leaf (in particular, a leaf petiole) and biomass of plants or morphological traits of under-ground plant parts. Biomass of plants of *Aquilegia* species and petiole parameters (diameter, the area of petiole and xylem cross-section, quantity of the conducting bundles) correlate with root volume and hypocotyl thickness.

The area of the petiole sclerenchyma is positively correlated with the plant diameter, the leaf petiole diameter and its cross-sectional area, and with the area of conducting bundles. The greater the plant leaf surface and its hydraulic conductivity, the better the development of mechanical tissues, which ensure an optimal location of photosynthetic organs in relation to sunlight and their supporting function.

A larger area of conducting bundles leads to a greater hydraulic conductivity of leaf petioles, which results in a higher absorption of water with dissolved minerals and formation of a larger biomass of a plant. A higher value of stomatal index results in a more efficient evaporation of water, which ensures cooling down of the leaf at higher air temperatures. The increase in the stomatal index (SI) was accompanied by an increase in the weight of a dry leaf, because a greater volume of evaporation intensifies a suction force. This brings a greater volume of water containing dissolved minerals to photosynthetic organs, which facilitates formation of more synthesizable substances.

Plants of *Aquilegia* species are labile and able to adapt to the conditions of the introduction region due to the features of anatomical structure of their vegetative organs – the presence of interfascicular cambium, which under certain conditions can form additional conducting bundles, mechanical tissue and petiole parenchyma.

The ratio of the petiole diameter to its length (dp/Lp) is a trait that determines the adaptation of *Aquilegia* species to the conditions of the Ukrainian steppe. First, this ratio correlates with many morphological and anatomical traits of the leaf. Second, it has a close significant correlation with the area of conducting tissues of the petiole, which determines its hydraulic conductivity. For *Aquilegia* species, increased area of petiole vessels (hence, the hydraulic conductivity of the petiole) was accompanied by decreasing dp/Lp. The obtained dependencies are not universal, for example, a greater hydraulic conductivity of petiole of *Corylus colurna* is linked with a higher value of the area of conducting tissue (Netsvetov, 2012).

Effects of climatic factors on leaf anatomy and ecophysiology and root morphology

Species of plants have a set of phenotypic characteristics determined by their genotype. These features either become important for adaptation to new growth conditions or do not play any role in adaptation to these conditions. The eco-geographical origin of species has a significant impact on the success of their adaptation to a new environment.

The species of *Aquilegia* from warmer habitat differ from other studied species by a long leaf petiole, larger area of conducting tissues, weight per unit of leaf area and dissection of the leaf plate. These traits of a leaf are typical for the species in the conditions of their natural habitats and foster their adaptation to the conditions of the Ukrainian steppe. It is well known that adaptive traits of relative growth rate, biomass gained, and specific leaf area have often been positively linked with greater reproductive output (Wright et al., 2004). In the Ukrainian steppe conditions, *Aquilegia* species from humid habitats are characterized by a high value of the leaf palisade index and a thick lower epidermis. The *Aquilegia* species from the regions with a low annual evaporation (Ev) develop a long root system to adjust to the extraction of water from deeper soil layers. The adaptive traits of these species are a thick upper epidermis for the accumulation of water, a lager thickness of columnar mesophyll, where an intensive synthesis assimilates, and a large number of conducting bundles in the leaf petiole to increase its hydraulic conductivity. These traits are necessary for the sustenance of the plant in a changing environment.

Conclusion

For the *Aquilegia* species of different eco-geographical origin, we detected the most important traits for adaptation to the Ukrainian steppe. For Balkan mountain forest species, these traits are a vigorous root system, a wide leaf petiole and a higher stomatal index. For sub-alpine and alpine species of Europe, the traits are a deep root system; a large number of adventitious roots; a higher stomata density and a small size of stomata; a large thickness of columnar mesophyll and leaf epidermis, and a higher palisade index; a large number of conducting bundles in the petiole. For the highland forest species of North America, the traits are a higher palisade index and a significant development of conducting bundles. The species of lowland forests of the Far East develop a thicker lower epidermis.

We determined new adaptive traits of a successful introduction of *Aquilegia* species and of their groups by their eco-geographical origin. For the individual species, it is the ratio of the petiole diameter to its length (dp/Lp), low values of which indicate a higher adaptation of species to new conditions. For the groups of species, these traits are volume of the root system, hypocotyl thickness, area of petiole xylem and the stomatal index.

We found out the relationships of the biomass of plants of *Aquilegia* species with the root volume, the hypocotyl thickness and the petiole diameter. Morphological and anatomical traits of the leaf are linked with the parameters of the root system of plants. The leaf area and thickness, the thickness of the columnar mesophyll correlate with the number and the length of adventitious roots. The area of petiole and its conducting bundles are linked with the hypocotyl thickness and the root volume.

The *Aquilegia* species from warmer regions have a bigger biomass, a higher number of regeneration buds and a greater petiole length. The species from colder habitats have a tendency to develop a deep root system, thicker columnar mesophyll and upper epidermis, and to form a large number of conducting bundles in petiole. The species from the habitat with less annual precipitation develop bigger thickness of the spongy mesophyll and size of the stomata, smaller thickness of the lower epidermis and palisade index. In the species from more humid habitats, we observed a tendency to form a root system with smaller diameter and volume, and a thicker lower epidermis. The plants from the regions with a low annual evaporation develop a deep root system, a thicker epidermis, a greater columnar mesophyll and a large number of conducting bundles in the petiole. Consequently, the climatic factors of the natural habitat of species affect the anatomical and morphological traits of their vegetative organs. Some of these traits become useful for adaptation of plants to the new growth conditions.

References

Agroclimatic world atlas, 1972. Gidrometeoizdat, Moscow, Leningrad, Russia (in Russian).

Bastida J.M., Alcantara J.M., Rey P.J., Vargas P., Herrera C.M., 2010. Extended phylogeny of *Aquilegia*: the biogeographical and ecological patterns of two simultaneous but contrasting radiations. Plant Systematics and Evolution 284, 171-185.

Bissing D.R., 1982. Evolution of leaf architecture in the chaparral species *Fremontodendron californicum* ssp. *californicum* (Sterculiaceae). American Journal of Botany 69 (6), 957-972.

Boyne R.L. 2011. Comparative leaf anatomy of invasive and noninvasive climbers under different light levels: Implications for ecophysiological performance and phenotypic plasticity. B.Sc. Thesis, Queensland University of Technology, Brisbane, Australia.

Boyne R.L., Osunkoya O.O., Scharaschkin T., 2013. Variation in leaf structure of the invasive Madeira vine (*Anredera cordifolia*, Basellaceae) at different light levels. Australian Journal of Botany 61, 412-417.

Brodribb T.J., Jordan G.J., Carpenter R.J., 2013. Unified changes in cell size permit coordinated leaf evolution. New Phytologist 199, 559-570.

Buinova M.G., 1988. Anatomy and pigments of plant leaves of Transbaikalia, Nauka, Novosibirsk, Russia (in Russian).

Ceulemans R., Impens I., Lemeur R., Moermans R., Samsuddin Z., 1978. Water movements in the soil-poplar-atmosphere system. Oecologia Plantarum 13 (1), 1-12.

Crawford R.M., 1989. Studies in plant survival. Ecological case histories of plant adaptation to adversity, Blackwell, Oxford, 296 p.

Dyachenko A.P., 1978. Comparative analysis of structural and functional features of the photosynthetic apparatus of different ecological groups of higher plants. Mesostructure and functional activity of the photosynthetic apparatus. Sverdlovsk, Russia (in Russian).

Eamus D., Prichard H., 1998. A Cost-Benefit Analysis of Leaves of Four Australian Savanna Species. Tree Physiology 18, 537-545.

Esau K., 1980. Anatomy of Seed Plants, 2nd Ed, John Wiley & Sons, Inc., New York.

Funk J.L., 2013. The physiology of invasive plants in low-resource environments. Conservation Physiology 1, 1-17.

Franks P.J., Beerling D.J., 2009. Maximum leaf conductance driven by CO_2 effects on stomatal size and density over geologic time. Proceedings of the National Academy of Sciences (PNAS) 106(25), 10343-10347.

Gianoli E., Valladares F., 2012. Studying phenotypic plasticity: The advantages of a broad approach. Biological Journal of the Linnean Society 105, 1-7.

Gupta B. 1961. Correlation of tissues in leaves II. Absolute stomatal numbers. Annals of Botany 25, 71-77 .

Gyorgy E., 2009. Anatomic adaptive strategies of some Cormophytes with individuals growing in light and shaden conditions. Notulae Botanicae Horti Agrobotanici Cluj-Napoca 37(2), 33-39.

Holland N., Richardson A.D., 2009. Stomatal length correlates with elevation of growth in four temperate species. Journal of Sustainable Forestry 28, 63-73.

Hui F., Guixiang Y., Te C., Leyi N., Meng Zh., Shengrui W., 2012. An alternative mechanism for shade adaptation: implication of allometric responses of three submersed macrophytes to water depth. Ecological Researches 27, 1087-1094

Klein R.M., Klein D.T., 1974. Methods of plant research, Kolos, Moscow, Russia (in Russian).

Krokhmal I., (2013). Functional anatomy of leaf *Campanula alliariifolia* Willd. 2013. Notulae Botanicae Horti Agrobotanici Cluj-Napoca 41(2), 388-395.

Menshakova M.Y., Zhirov V.K., Haitbaev A.H., Gaynanova R.I., 2008. Variability of the photosynthetic apparatus of plants: boreal and subarctic ecosystems, Nauka, Moscow, Russia (in Russian).

Netsvetov M.V., 2012. Influence of wind onto the structure and biomechanics of leaves *Corylus colurna* (Betulaceae). Botanicheskiy Zhurnal 97(2), 160-173.

Nicotra A.B., Atkin O.K., Bonser S.P., Davidson A.M., Finnegan E.J., Mathesius U., Poot P., Purugganan M.D., Richards C.L., Valladares F., van Kleunen M., 2010. Plant phenotypic plasticity in a changing climate. Trends in Plant Science 15, 684-692.

Niklas K.I., 1994. Plant allometry: the scaling of form and process. University of Chicago Press, Chicago.

Nikolaevskaya E.V., 1990. Variability of morphological and anatomical structure of leaves of different ecotypes of *Trifolium repens* L. in connection with the vertical zoning. Bulletin LGU 3, 4 (24), 33-44.

Osunkoya O.O., Bayliss D., Panetta F.D., Vivian-Smith G., 2010. Leaf trait coordination in relation to construction cost, carbon gain and resource-use efficiency in exotic invasive and native woody vine species. Annals of Botany 106, 371-380.

Osunkoya O.O., Boyne R., Scharaschkin T., 2014. Coordination and plasticity in leaf anatomical traits of invasive and native vine species. American Journal of Botany 101 (9), 1423-1436.

Poorter H., Lambers H., Evans J. R., 2014. Trait correlation networks: A whole-plant perspective on the recently criticized leaf economic spectrum. New Phytologist 201, 378-382.

Poorter H., Remkes C., 1990. Leaf area ratio and net assimilation rate of 24 wild species differing in relative growth rate. Oecologia 83, 553-559.

Roy B.A., Stanton M.L., Eppley S.M., 1999. Effects of environmental stress on leaf hair density and consequences for selection. Journal of Evolutionary Biology 12, 1089-1103.

Sack L., Cowan P.D., Jaikumar N., Holbrook N.M., 2003. The 'hydrology' of leaves: Co-ordination of structure and function in temperate woody species. Plant, Cell & Environment 26, 1343-1356.

Sack L., Scoffoni C., 2013. Leaf venation: Structure, function, development, evolution, ecology and applications in the past, present and future. New Phytologist 198, 983-1000.

Scheepens J.F., Frey E.S., Stocklin J., 2010. Genotypic and environmental variation in specific leaf area in a widespread Alpine plant after transplantation to different altitudes. Oecologia 164, 141-150.

Tsialtas J.T., Kassioumi M., Veresoglou D.S., 2002. Leaf Construction Cost of the Most Abundant Species in an Upland Grassland Area of Northern Greece. Russian Journal of Plant Physiology 49 (3), 360-363.

Vasilevskaya V.K., 1954. Leaf forming of drought-resistant plants. Turkmenian Academy of Sciences, Ashgabat.

Vogel S., 2009. Leaves in the lowest and highest winds: temperature, force and shape. New Phytologist 183, 13-26.

Wright I.J., Reich P.B., Westoby M., Ackerly D.D., Barush Z., Bongers F., Cavender-Bares J., Chapin F.S., Cornelissen J.H.C., Diemer M., Flexas J., Garnier E., Groom P.K., Gulias J., Hikosaka K., Lamont B.B., Lee T., Lee W., Lusk C., Midgley J.J., Navas M-L., Niinemets U., Oleksyn J., Osada N., Poorter H., Poot P., Prior L., Pyankov V.I., Roumet C., Thomas S.C., Tjoelker M.G., Veneklaas E., Villar R., 2004. The worldwide leaf economics spectrum. Nature 428, 821-827.

Zhongqiang L., Dan Y., 2009. Factors affecting leaf morphology: a case study of *Ranunculus natans* C. A. Mey. (*Ranunculaceae*) in the arid zone of northwest China. Ecological Researches 24, 1323-1333.

Zhang S.B., Guan Z.J., Sun M., Zhang J.J., Cao K.F., Hu H., 2012. Evolutionary association at stomatal traits with leaf vein density in *Paphiopedilum*, Orchidaceaea. PLOS ONE 7(6), e40080.