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RESEARCH ARTICLE

## Morpho-anatomical adaptation of the leaves of certain *Veronica* species to arid conditions

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### Abstract

This study's objective was to evaluate the adaptive potential of the leaves of *Veronica* (*V. austriaca*, *V. incana*, and *V. prostrata*) in the arid conditions of the Kryvyi Rih Botanical Garden NAS of Ukraine (KBG) located in the Right-Bank Steppe Cisdnieper. Leaves have been obtained from the collection of ornamental plants of KBG, where these plants are introduced for ten years. The seasonal dynamics of leaf development have been studied during the growing season of 2018, when the most arid climatic conditions were observed.

The leaves of the investigated species were found to be dorsoventral, amphistomatic, with an anomocytic stomatal apparatus and bifacial mesophyll. Changes in the anatomical structures of the leaf blade towards xeromorphism were determined. In particular, in all studied specimens thickening of the cuticle, adaxial and abaxial epidermises (except *V. prostrata*, where the upper epidermis decreased by 35 %) mesophyll occurred. The number of stomata on the abaxial epidermis in all species decreased (the most notably in *V. prostrata*). Such increase of xeromorphism in *V. austriaca*, *V. incana*, and *V. prostrata* is an adaptive reaction of plants to the effects of extreme arid weather and climatic conditions of the Kryvorizhzhya, which testifies to the plasticity of investigated species and their high adaptation potential to the climatic conditions of the Right-Bank Steppe Cisdnieper.

**Keywords:** *Veronica*, anatomy, leaf, plasticity, drought resistance

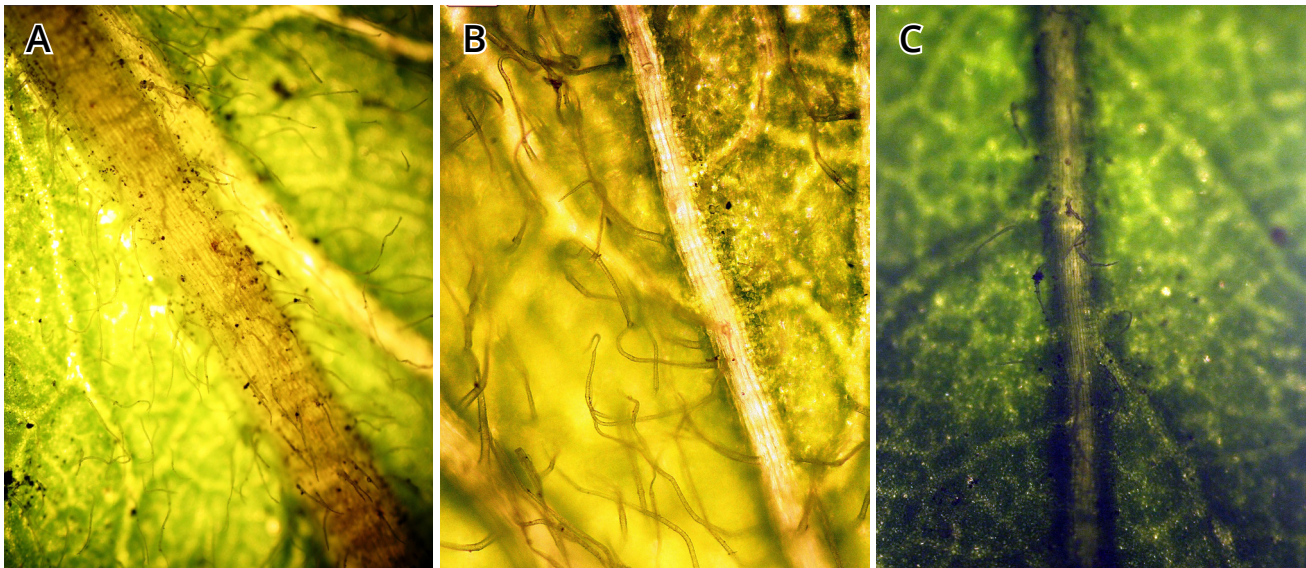
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### Introduction

In the conditions of increasing technogenic pressure in Ukraine and global climate warming, it is necessary to search for new drought- and pollution-resistant species for purposes of ornamental planting. According to the long-term introductory observations of the *Veronica* L. representatives in the collection of the Kryvyi Rih Botanical Garden of the NAS of Ukraine (KBG), five species (*V. armenica* Boiss., *V. austriaca* L., *V. incana* L., *V. prostrata* L., and *V. spicata* L.) were identified as promising to replenish the cultural flora of the Right-Bank Steppe Cisdnieper. These species occur in

different conditions in the wild nature. They came from Circumboreal, Irano-Turanian, and Eastern Asiatic floristic regions (Yelenevskiy, 1978; Takhtajan, 1978; Kotov et al., 1999). In ornamental gardening, these unpretentious plants are appreciated for the extraordinary beautiful flowers, long-blooming, various shapes and color of leaves, and high resistance to diseases and pests. Unfortunately, in the Kryvyi Rih plantings, these species are not used due to insufficient information regarding their ecological preferences and biological characteristics (Chypylyak et al., 2014).

Investigations of the morphological and anatomical structure of the plant organs,



**Figure 1.** Trichomes of *Veronica austriaca* (A), *V. incana* (B), and *V. prostrata* (C) leaves (×4).

particularly the leaves (as the most labile to the influence of environmental factors), are principal for determination of the species' adaptive capacity (Grodzinskiy, 2013; Shvets, 2013).

Some morpho-anatomical studies of *Veronica* from different growth areas were performed to standard medicinal raw materials (Kaplan et al., 2007; Marchyshyn et al., 2015; Mocan et al., 2015; Antsyshkina et al., 2017). However, information on the anatomic and morphological structure of *Veronica* leaves in the context of adaptive plasticity to the conditions of the Right-Bank Steppe Cisdnieper is absent.

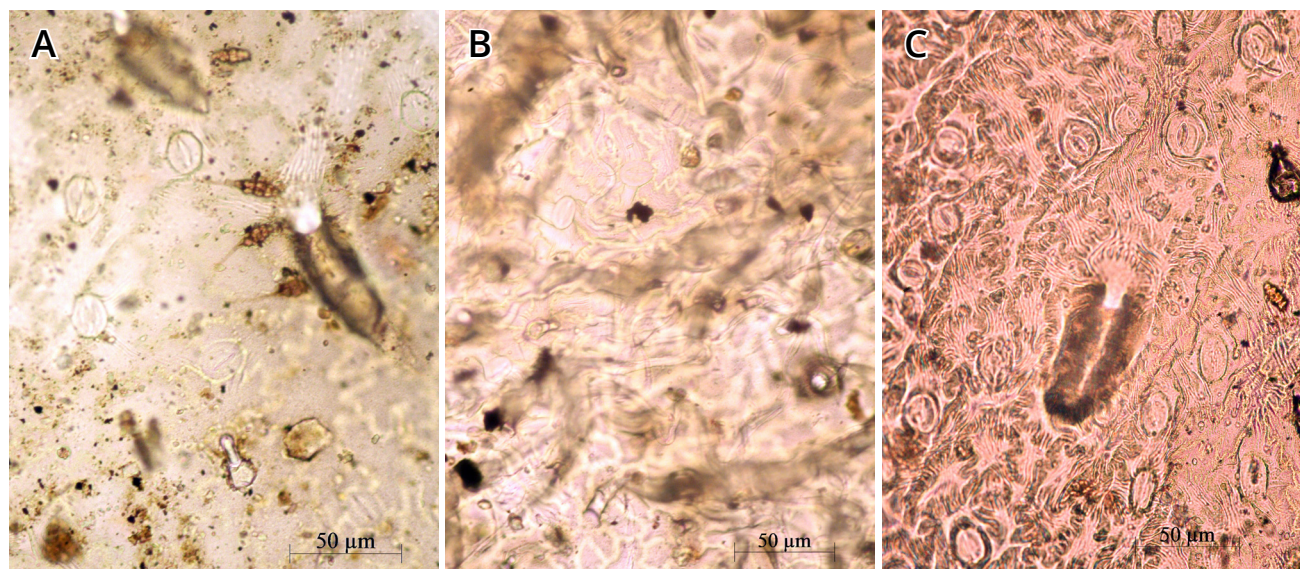
Three *Veronica* species (*V. austriaca*, *V. incana*, and *V. prostrata*) with different biomorphological and decorative features (Leshcheniuk, 2017, 2018, 2019) that were introduced at the KBG for over ten years were selected for the current research. Under the introduction conditions, *V. incana* and *V. prostrata* belong to the hamephytes, and *V. austriaca* to hemicryptophytes (Raunkiaer, 1907). All species are herbaceous polycarpics (Serebryakov, 1964).

## Material and methods

The research was conducted in KBG during the growing season of 2018. That year was characterized by the extreme weather-climatic conditions. The leaves were selected twice during the growing season – in the first

ten-day period of June during the completion of the spring regrowth phase (summer sampling), and in the third ten-day period of September at the beginning of completion of vegetative development (autumn sampling). Sampling in the autumn was preceded by high temperature during June–August (from 32.5 to 35.3°C) and the lack of precipitation for a long time. During summer of 2018, only 71.6 mm of precipitations fell, which is twice less than the usual precipitation (160 mm) during this period in Kryvyi Rih.

We used temporary slides made from mature leaves to study their anatomical and morphological structure. Median leaves formation of 15 plants per each species was used (Vekhov et al., 1980; Barykina et al., 2004). Cross-sections were prepared by hand with a razor on the level of a third of the leaf's length. The slides were examined with Carl Zeiss Primo Star microscope at ×10, ×20, and ×40 magnifications. The leaf, epidermis, and mesophyll thickness was measured at the same distance from the edge of the leaf and the main vein. The number of stomata was counted per the field of view. Stomatal apparatus has been classified following Baranova (1985). General morphological characteristics of the leaves were provided following Zyman et al. (2004). Measurements were performed in the AxioVision 4.8.2 environment. Data were statistically processed through the dispersal analysis, according to Dospekhov (1985) using MS Excel 2003.



**Figure 2.** Adaxial epidermis of *Veronica austriaca* (A), *V. incana* (B), and *V. prostrata* (C) leaves.

The introduction area (Kryvorizhzhya) is located in the southwest of the Dnipropetrovsk region, in the Steppe natural zone. It belongs to Ukraine's arid regions (Kazakov et al., 2005), where the average precipitation is 425–450 mm per year (with a maximum in early summer). The rest of the summer is characterized by prolonged droughts, high anomalous temperatures, and little or absent precipitations. In the warm period, dry winds are observed. The winter has harsh climatic conditions with icing, water stagnation on the soil surface, and frequent thaws followed by short but low freezing temperatures ( $-27 - -30^{\circ}\text{C}$ ). Due to global warming in recent decades, some meteorological indicators have changed in the Kryvorizhzhya (Kazakov et al., 2005; Rp5, 2020). The average annual temperature increased from  $+8.5^{\circ}\text{C}$  in 1987 to  $+10.0 - +11.5^{\circ}\text{C}$  in 2014–2018. The average daily temperature in January–February increased from  $-5.1 - -4.4^{\circ}\text{C}$  in 1987 to  $-1.7 - -1.5^{\circ}\text{C}$  in 2018. The average daily temperature of the hottest months, July and August, also increased from  $+22 - +21^{\circ}\text{C}$  in 1987 to  $+23.5 - +24.8^{\circ}\text{C}$  in 2018. In the summer, there are prolonged droughts in the presence of anomalously high temperatures ( $+36.7 - +38.1^{\circ}\text{C}$ ). In general, the climatic conditions of 2018 were hot and extreme for growing plants. The increased temperatures were observed from May (daytime temperature reached  $+32.5 - +33.3^{\circ}\text{C}$ ) till September (with temperature maximum of  $+35.3^{\circ}\text{C}$ ). The annual precipitation in 2018 was only 364 mm. Only a

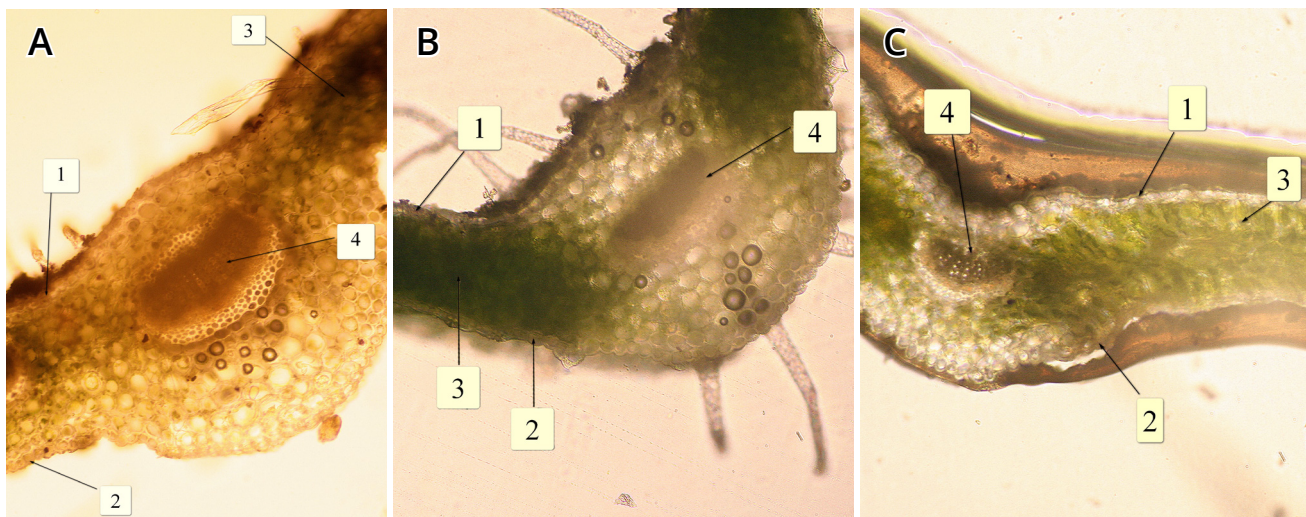
few rains fell from April to October, which led to prolonged air and soil drought during the vegetation period.

## Results and discussion

The leaves of the middle formation of *V. austriaca* have ovate or ovate-oblong leaf blade, with a wedge-shaped or rounded base, with a toothed margin and a pointed tip,  $3.7 \pm 0.2$  cm long, and  $1.7 \pm 0.2$  cm wide. The leaf blade of *V. incana* is lanceolate, small-toothed,  $5.9 \pm 0.9$  cm long, and  $1.8 \pm 0.2$  cm wide. The leaf blade of *V. prostrata* is oblong-lanceolate or linear-lanceolate, with a wedge-shaped base, crenate margin, and obtuse apex,  $2.2 \pm 0.4$  cm long, and  $0.5 \pm 0.1$  cm wide.

The leaf blade of all studied species is dorsoventral. The leaves' upper and lower surfaces are covered by single- and multicellular covering trichomes, at the base of which two or three-celled rosettes are located. In *V. austriaca* there are also four-celled rosettes at the base of trichomes. The longest covering trichomes having sharp tips are bent to the leaf surface. The highest number of trichomes has been observed in *V. incana* (Fig. 1). In all investigated species, besides covering trichomes, there are also glandular hairs. In *V. prostrata* they are clavate, with a unicellular elongated stem and bicellular head.

The leaves' upper and lower epidermises consist of a single layer of cells with highly convoluted walls. The adaxial epidermis cells,



**Figure 3.** Cross-section of *Veronica* leaf blade in the central vein area: **A** – *V. austriaca*; **B** – *V. incana*; **C** – *V. prostrata* (×20). 1 – adaxial epidermis; 2 – abaxial epidermis; 3 – mesophyll; 4 – central vein.

which are covered with a cristate cuticle, are smaller than those located on the abaxial surface (almost 50% in the *V. austriaca*, and *V. incana* and 21% – in the *V. prostrata*) (Table 1; Fig. 2). The leaves of the investigated species are amphistomatic, usual for xerophytes and mesophytes that grow in arid conditions (Vasilevskaya, 1965). The stomata of the anomocytic type, oval or spherical-oval, granose, located randomly on the adaxial and abaxial sides of the leaf blade on the same level as other epidermal cells. Same as other epidermal cells, stomata are also smaller on the adaxial surface of the leaf blade.

The mesophyll is bifacial. Palisade parenchyma, which consists of 2–3 layers of elongated cells, is located under the upper epidermis.

Below the palisade cells, a two- or three-layered spongy parenchyma is formed by ovate cells, which are located loosely in early summer and more densely in the autumn. Among the mesophyll cells, idioblasts with calcium oxalate raphides are scattered. Such raphides have a protective function and help to scatter light, which protects plants against excessive insolation and overheating (Nakata, 2003; Franceschi & Nakata, 2005; López-Macías et al., 2019). Open collateral vascular bundles are accompanied by the parenchymatic lining (Fig. 3).

Due to the temperature stress and prolonged air-soil drought in 2018, quantitative and functional changes in the leaf blade's anatomical structures of investigated plants

occurred. *V. austriaca* has undergone the slightest changes compared to other species. The thickness of the leaf blade in *V. austriaca* from the beginning of summer to the end of September increased by 8%, and mesophyll – by 6%. The thickness of the cuticle and the adaxial epidermis in *V. austriaca* increased the most (by 12 and 11%, respectively). At the same time, the thickness of the abaxial epidermis in this species increased only by 5%. The size of stomata on the adaxial surface during the growing season did not change significantly. However, the size of the stomata on the abaxial surface decreased by 8%. The number of stomata did not change significantly (Table 1).

Among the investigated species, the most significant thickening of the cuticle (40%), adaxial (60%), and abaxial (27%) epidermises was recorded in *V. incana* plants (Table 1). The thickness of the leaf blade and mesophyll increased by 20%. By the end of the growing season, palisade mesophyll obtained a more compact structure. The size of stomata on the adaxial surface increased by 8%. However, the stomata on the abaxial side became much smaller by 13%. The number of stomata on the abaxial epidermis simultaneously decreased by 38%.

*Veronica prostrata* demonstrated certain features of adaptation to the prolonged hot and dry period. At the beginning of summer, the mesophyll in this species had no clear distinction between palisade and spongyform parenchymas and was represented by loosely placed cells. However, at the end of the

**Table 1.** Morphometric parameters of the leaf blade in the investigated *Veronica* species.

Parameters	Sampling time	<i>V. austriaca</i>		<i>V. incana</i>		<i>V. prostrata</i>	
		M ± m	CV, %	M ± m	CV, %	M ± m	CV, %
The thickness of the leaf blade, µm	summer	184.5 ± 5.0	12.3	203.1 ± 3.7	9.1	284.0 ± 5.7	10.6
	autumn	200.9 ± 7.3	18.2	245.3 ± 3.6	7.4	385.3 ± 9.7	12.6
Cuticle thickness, µm	summer	4.8 ± 0.5	25.4	3.9 ± 0.2	22.3	7.5 ± 0.3	20.7
	autumn	5.4 ± 0.2	21.5	5.4 ± 0.3	20.1	8.1 ± 0.6	22.6
The thickness of adaxial epidermis, µm	summer	8.9 ± 0.8	15.4	9.0 ± 0.3	15.3	18.5 ± 0.8	20.9
	autumn	10.4 ± 0.3	12.3	14.4 ± 0.6	21.2	11.7 ± 0.3	11.4
The thickness of abaxial epidermis, µm	summer	16.8 ± 0.9	15	17.3 ± 0.6	17.1	23.3 ± 0.7	14.8
	autumn	17.6 ± 0.6	13.4	22.0 ± 0.6	12.9	23.1 ± 0.3	6.1
The thickness of the mesophyll, µm	summer	131.3 ± 1.9	7.1	146.9 ± 2.3	7.9	199.6 ± 4.1	10.3
	autumn	139.7 ± 3.3	11.9	172.0 ± 2.3	6.7	278.4 ± 9.6	17.3
Sizes of stomata on adaxial surface (length / width), µm	summer	28.8 ± 0.7 / 19.7 ± 0.8	11.4 / 9.6	24.9 ± 0.4 / 20.3 ± 0.3	8.7 / 6.7	26.9 ± 0.2 / 25.8 ± 0.3	9.5 / 11.4
	autumn	29.7 ± 0.5 / 20.1 ± 0.4	9.6 / 7.6	27.2 ± 0.1 / 21.8 ± 0.4	5.7 / 7.9	26.3 ± 0.9 / 24.1 ± 0.1	11.7 / 9.9
Sizes of stomata on abaxial surface (length / width), µm	summer	30.8 ± 0.7 / 21.9 ± 0.5	13.5 / 9.6	27.5 ± 0.5 / 17.6 ± 0.5	11.3 / 11.2	29.8 ± 0.5 / 22.2 ± 0.6	8.5 / 13.8
	autumn	28.4 ± 0.5 / 22.4 ± 0.4	9.6 / 8.5	23.9 ± 0.2 / 17.1 ± 0.4	5.1 / 10.8	24.3 ± 0.7 / 18.1 ± 0.4	13.6 / 11.9

**Note.** **summer** – first ten days of June; **autumn** – last ten days of September; **M ± m** – the arithmetic mean and standard deviation; **CV** – coefficient of variation.

growing season, the mesophyll had a clear differentiation into 2–3-layered palisade parenchyma with elongated, tightly arranged, cylindrical cells and 2–3-layered spongy parenchyma constructed by roundish cells. The thickness of the mesophyll and the leaf blade in general increased by 35% (Table 1), which is the most notable among the studied species. The cuticle in *V. prostrata* thickened only by 8%, but its size was the largest among the species studied both in early summer and autumn – 7.5 and 8.1 µm, respectively. The adaxial epidermis, unlikely to other species, became 35% thinner. The number of stomata decreased by 77%, their size on the adaxial surface – decreased by 7%, and on the abaxial surface – by 18%. We believe that such changes in the anatomical structure of leaf blade are an adaptive response of plants to the effects of hot and dry conditions and contribute to the regulation of gas exchange, protection of plants from overheating and excessive transpiration (Esau, 1969).

## Conclusions

The leaves' anatomical and morphological features may indicate adaptation of *V. austriaca*, *V. incana*, *V. prostrata* species to the arid climate of Kryvorizhzhya. In all investigated species, the increase of xeromorphic features (thickening of the leaf blade, mesophyll, cuticle, and epidermis, as well as changes of the stomatal indices and the arrangement of mesophyll cells) has been observed.

The most significant changes in the anatomical structure of the leaf were observed in *V. prostrata*. *Veronica incana* was characterized by the most numerous trichomes and the most significant thickening of the cuticle, adaxial and abaxial epidermis. The slightest changes in the anatomical structure of the leaf blade were observed in *V. austriaca* plants. The revealed changes in the leaf structure of the investigated *Veronica* species can be considered an adaptive response of plants to the effects of extreme

arid climatic conditions. This indicates the plasticity of the investigated *Veronica* species and their adaptation potential to the conditions of the Right-Bank Steppe Cisdnieper.

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## Морфо-анатомічна адаптація листків окремих видів *Veronica* до посушливих умов

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З метою визначення адаптаційного потенціалу досліджено морфо-анатомічну структуру листка трьох видів роду *Veronica* (*V. austriaca*, *V. incana* та *V. prostrata*) за умов вирощування у Криворізькому ботанічному саду НАН України (КБС), що розташований у кліматичних умовах Правобережного степового Придніпров'я. Матеріал отримали з колекційного фонду квітничково-декоративних рослин КБС, де згадані види інтродуковано впродовж десяти років. Сезонну динаміку вивчали протягом вегетаційного періоду 2018 року, який характеризувався найпосушливішими умовами.

Виявлено, що рослини досліджуваних видів добре пристосовані до зростання в посушливих і спекотних умовах, про що свідчать ксероморфні риси анатомічної будови листків, зокрема: розвинуті опушення та кутикула, рафіди оксалату кальцію, амфістоматичний тип листової пластинки, а також диференційований мезофіл. Виявлено, що в умовах дефіциту вологи та за високої температури у рослин *V. austriaca*, *V. incana* та *V. prostrata* відбувалося посилення ознак ксероморфності, а саме: потовщення шару кутикули й обидвох епідермісів (за винятком *V. prostrata*), зменшення розмірів продихів та їхньої кількості на нижньому епідермісі листової пластинки, ущільнення клітин палісадного та губчастого мезофілів. Такі кількісні й якісні зміни структури листової пластинки можна розглядати як пристосувальну реакцію рослин на дію екстремальних посушливих погоднокліматичних умов, що свідчить про пластичність досліджених видів та їхній високий адаптаційний потенціал в умовах Правобережного степового Придніпров'я.

**Ключові слова:** *Veronica*, анатомія, листок, пластичність, посухостійкість