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Adaptive Features of the Leaf of *Juno vicaria* (Vved.) T. Hall & Seisums in Different Ecological Conditions of Uzbekistan

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Laboratory Anatomy and Cytoembryology of the Tashkent Botanical Garden Named after Academician F.N. Rusanov, Institute of Botany of the Academy of Sciences, Tashkent, Uzbekistan
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Abstract

The anatomical structure of the leaf of the Central Asian endemic species of *Juno vicaria*, growing in two different ecological conditions (Baysun Mountains of Surkhandary region and Tashkent Botanical Garden of Uzbekistan) was analyzed and diagnostic signs were determined. Also identified are adaptive mesomorphic and xeromorphic signs from an environmental point of view. A species that grows in natural habitat is dominated by more xeromorphic signs: a thickened outer wall of the epidermis; numerous epidermal, spongy cells; submerged stomata; small, multi-row sclerenchymal and collenchymal cells, which indicate a more xeromorphic and adaptability, as well as the wide distribution of this species to the natural growing conditions. In a species growing under the conditions of introduction of the Tashkent Botanical Garden, more mesomorphic features predominate: thin-walled, large, few epidermal, spongy cells; not immersed stomata; large non multi-row sclerenchymal and collenchymal cells, which show that the species is adapted to altered habitat conditions.

Keywords

Anatomy, Leaf, *J. vicaria*, Surkhandarya Region Baysun Mountains, Introduction, Tashkent Botanical Garden, Uzbekistan

1. Introduction

The genus *Iris* L. is the most polymorphic in the family of the same name with more than 200 species [1]. In the flora of Uzbekistan, this genus includes more than 30 species. Central Asia is one of the centers of diversity of species of Juno

irises (subgenus *Scorpiris* Spach). According to the latest F.O. Khassanov and N.K. Rakhimova [2] subgenus *Scorpiris* in the flora of Central Asia is represented by at least 30 species. K. Sh. Tojibaev *et al.* [3] described new species—*Iris austrotschatkalica* Tojibaev, F. Karim. & Turgunov from the Chatkal Range of the Fergana Valley and Iris khassanovii Tojibaev & Turgunov from the Gissar Range.

F. O. Khasanov *et al.* [4] described two species—*Iris rudolphii* F.O. Khass., Esankulov et Achilova and *Iris victoris* F.O. Khass. U. Khuz. & N. Rakhimova, from the territory of Uzbekistan (Kelif-Sherabadskaya ridge). G.A. Lazkov *et al.* [5] described a new species—*Iris rodionenkoi* Lazkov et Naumenko from Kyrgyzstan. To date, representatives of the subgenus *Scorpiris* in Central Asia have at least 36 species. According to B. Mathew [6], representatives of the subgenus *Scorpiris* on the globe have no more than 55 species, and that Central Asia is the center of origin of Juno irises.

In the literature data, the structure of the epidermis and leaf cuticle is given special attention. Some environmental factors can affect the structure of the epidermis, without any connection with the genotype. For example, many authors believed that the size and frequency of stomata depend on environmental conditions [7]-[11]. T. Bacic [12] showed that the number and size of stomata varied depending on the influence of light and the age of the plants. T. Nikolic [13] leads to the phenotypic plasticity of the width of epidermal cells caused by different lighting conditions. According to J. Pazourek [14], the stomatal frequency decreases with decreasing light intensity. G.N. Knecht and J. W. O'Leary [15] observed the development of a larger number of stomata with increased light intensity, T.W. Lucansky and K.D. Clough [16] pointed out the dependence of the anatomical features of the leaf on the xeromorphic and mesomorphic of the habitat.

Chinese and English scientists Qi-Gen Wu & D. F. Cutler [17] described the taxonomic, evolutionary and ecological significance of the anatomy of leaves of rhizomatous 113 species and 20 intraspecific taxa from the first three subgenera Iris. The taxonomic and ecological significance of the structural sings was evaluated. The relationships and evolutionary position of these taxa are discussed. Some species with uncertain taxonomic position are discussed and their classification based on anatomical data is proposed. A correlation between certain anatomical sings and the type of habitat for certain types of irises is shown. Also divided into 3 groups based on xeromorphic, mesomorphic and gelomorphic sings of the leaf.

B. Mitic, T. Nikolic, Z. Liber [18] analyzed relationships in the alpine-dinaric and cultured populations of the genus Iris, a number of Pallidae. Some morphological and anatomical features of the leaf were investigated (length and width of stomata, length and width of epidermal cells, number of stomatal and epidermal cells per mm^2, and length of styloids). The results of cluster and PCA analysis showed that, based on the study of the anatomical features of a leaf of the Pallidae series, five main groups of populations were identified.

Researchers N. Kandemir, A. Çelik, F. Ullah *et al.* [19] studied the taxonomic significance of the anatomical features of a leaf of 10 species of the Iris subgenus Scorpiris Spach (*I. peshmeniana*, *I. aucheri*, *I. caucasica* ssp. Turcica, *I. caucasica* ssp. Caucasica, *I. pseudocaucasica*, *I. nezahatiae*, *I. galatica*, *I. persica*, *I. stenophylla* ssp. Margaretiae, *I. stenophylla* ssp. Stenophylla), native to Turkey. The anatomical features of a sheet of 10 taxa were studied in detail and divided into three groups. It was found that some anatomical features of a leaf have a taxonomic marker for differentiating taxa. *I. persica* is different from other proven taxa, however, two species (*I. peshmeniana* and *I. aucheri*) were closely related.

We have previously studied the morpho-anatomical structure of the vegetative organs of some species of *Iris* (*I. sogdiana, I. korolkowii, I. stolonifera, I. alberti*) and *Juno* (*J. svetlanae*) to identify the diagnostic features specific to each section (*Limniris* Tausch, *Iris* L., *Hexapogon* (Bunge ex Alek.) Baker and *Juno* Tratt. [20]-[25].

For the first time, we have previously studied the anatomical structure of the vegetative organs of the species *J. hippolyti* and *J. narbuti* was studied in the conditions of Kyzylkum (Uzbekistan, Province Bukhara) and revealed the diagnostic features of these species. A different combination of xeromorphic and mesomorphic features was determined, which ensures adaptation to habitat conditions [26].

2. Materials and Methods

The object of the study is the *Juno vicaria*—perennial bulbous species of the genus *Iris* L. of family Iridaceae. In nature, the species range covers the southwestern Pamir-Alai. Species identified by F.O. Khassanov *et al.* [2].

Juno vicaria **Vved.** Perennial. The roots are thickened, fusiform. Bulb 1 - 3 cm thick. The arrow is 20 - 40 cm tall, with leaves spread, internodes are noticeable. The leaves are light green, sickle-shaped, gradually narrowed to the apex, fringed along the edge, rough, lower 1.5 - 3 cm wide. Flowers in the number of 1 - 4, odorless. Perianth tube 4 - 4.5 cm long, purple, with darker veins. Outer tepals 4 - 5.5 cm long, light purple; nail with parallel edges, 10 mm wide, with 4 dark purple, branching veins, gradually turning into oblong, usually notched plate at the top 0.8 - 1.4 cm wide, 1.2 - 1.7 cm long, white or yellowish on the edge light purple and purple veins, with a dark yellow spot on the sides of the ridge; comb yellow or white or white with a yellow base, solid, curly. Internal tepals 2 - 2.5 cm long, purple, with darker veins, with sharp-rhombic or 3-lobed lamina. The blades of the branches of the column are light purple, with darker veins, obliquely triangular, obtuse, entire, 4 - 6 mm wide, 11 - 20 mm long. The stigma is semicircular, notched. Anthers and pollen are whitish. It grows on rocky slopes and between rocks in the lower mountain belt. General distribution. Central Asia (South-West Pamir-Alay). Ecology: In the middle belt of mountains. Area: Central Asia (South-Western Pamir Alai) [27].

Plant samples were collected from two different geographical habitats of Uzbekistan (in the natural conditions—growth of the Baysun Mountains of Surkhandarya region and conditions of introduction from the exhibition of oniony introduced plants of the Tashkent Botanical Garden) at the end of April and the beginning of May inthe phase of flowering 2016-2017 (**Figure 1**).

J. vicaria collected from the Surkhandarya region (Baysun mountains). It grows among perennials and shrubs on stony-fine-earth slopes of the southwestern Pamir-Alai. Endem. Baysun mountains (Surkhandarya region)—a mountain range in Uzbekistan, the south-western spur of the Gissar range. The length of the ridge is about 150 km, the maximum height is 4425 m. The average height is 2500 - 3000 meters. The range is composed of limestone, sandstones, and clays. On the slopes of the ridge starts the river Akdarya. The lower parts of the slopes are covered with semi-desert vegetation, above are forests of Juniper and alpine meadows [28].

J. vicaria in Tashkent Botanical Garden was introduced in 2010 with seeds and bulbs. Tashkent Botanical Garden is located in the north-eastern part of Tashkent in the basin; Chirchik belt at an altitude of 450 - 480 m above sea level. The climate is sharply continental. The average annual temperature of 13.8˚C. Precipitation falls mainly in the autumn-winter and spring periods; soil cultural-irrigated gray soils or irrigated the soil of desert and desert-steppe areas (sierozem) [29].

Samples of fresh plants were fixed in 70% alcohol solution.The leaf epidermis was studied on paradermal and cross sections. The cross sections of the leaf are made through the middle. Preparations prepared by hand were stained with methylene blue followed by gluing to glycerin-gelatin [30]. Descriptionsof the main tissues and cells are given according to K. Esau [31], N.S. Kiseleva [32],

(a) (b)

Figure 1. General view of *Juno vicaria*: (a) In the natural growing conditions (Boysun); (b) In the conditions of introduction (Tashkent Botanical Garden).

and the epidermis according to N.A. Aneli [33]. Anatomical measurements were made using a micrometer eyepiece fixed in a microscope. On a paradermal section, the number of adaxial and abaxial epidermis cells and stomata in 1 mm^2 surface area was counted. The length and width of the stomata were measured with a micrometer. Various anatomical features of the leaf (mesophyll structure, epidermis height, cuticle thickness, diameter of spongy parenchyma and collenchyma, diameter and number of vessels, stoma length and width, stomata submerged, number of layers of mesophyll cells, number of spongy parenchyma layers and collenchyma, type of conductive beams) as distinctive features. Photographs of micromorphological features of parts of the sheet were taken using a camera attached to a microscope *Motic B* 1-220A-3.

In a statistical analysis of 15 different leaf symbols (height of the epidermis, cuticle thickness, the number of adaxial and abaxial epidermis cells and stomata in 1 mm^2, the diameter of the spongy parenchyma and collenchyma, the diameter and number of vessels, the length-width of the stomata, the stomata embedded, the number of spongy parenchyma and collenchyma).Thirty measurements for each character were made; average value and the standard deviation values of these measurements were calculated. Statistical processing of quantitative data was carried out according to generally accepted criteria [34] using a personal computer (MS-Excel program). The results of statistical analysis are shown in Table 1 and Table 2.

3. Results and Discussion

Juno vicaria (Vved.) T. Hall & Seisums—leaves are light green, crescent-shaped, fringed along the edge, more or less rough.

Table 1. Quantitative indicators of the epidermis and stomata leaf *J. vicaria*, growing in two ecological conditions.

Place of growth	Показатель							
	Epiderma			Stoma				
	outer wall thickness, μm	number per 1 mm^2		length, μm	width, μm	immersion, μm	number per 1 mm^2	
		adax.	abax.				adax.	abax.
природа	5.3 ± 0.04	30.4 ± 0.32	60.8 ± 0.58	45.5 ± 0.41	40.9 ± 0.39	16.7 ± 0.17	–	186.5 ± 2.13
интродукция	6.9 ± 0.05	41.8 ± 0.42	72.2 ± 0.83	48.3 ± 0.40	63.3 ± 0.62	32.1 ± 0.33	–	148.8 ± 2.71

Note: – adax.—adaxial, abax.—abaxial epidermis. Values that are significantly different from those of the nature and introduction of these species are underlined.

Table 2. Quantitative indicators of leaf of *J. vicaria*, growing in different environmental conditions (n = 30).

Place of growth	Indicators							
	Epidermal cell height		Spongy parenchyma		Collenchyma		Vessels of conductive bundles	
	adax.	abax.	d-cells, μm	number of rows	d-cells, μm	number of rows	number of rows	number of rows
Nature	81.8 ± 1.43	21.1 ± 0.09	29.2 ± 0.13	7 - 8	7.2 ± 0.09	7 - 8	7 - 8	11.8 ± 0.08
Introduction	95.5 ±1.33	31.8 ± 0.31	36.6 ± 0.30	5 - 6	10.3 ± 0.14	8 - 9	7 - 8	22.2 ± 0.13

Note: - adax.—adaxial, abax.—abaxial epidermis. Values that are significantly different from those of the nature and introduction of these species are underlined.

In the paradermal section, the underlying epidermal cells can be attributed to a straight clan by the character of the boundary walls [33]. The adaxial epidermis cells belong to the rectangular-wall type of a straight clan, which are rectangular cells located along the vertical axis of the leaf. The cells of the abaxial epidermis belong to the rhombo-stenotic type of a rectilinear clan, which consist of a combination of straight wall and rhombus wall cells. The cells of the adaxial epidermis are larger than abaxial. On the adaxial and abaxial sides, the number of epidermal cells is 1 mm^2 more in a species growing under the conditions of introduction (41.8 ± 0.42 - 72.2 ± 0.83), less in natural conditions (30.4 ± 0.32 - 60.8 ± 0.58) (**Figure 2**, **Table 1**).

In the course of the research it was found that in the studied species the leaves are hypostomatic—the stomata are located only in the epidermis of the abaxial side of the leaf. The stomata are transverse to the longitudinal axis of the sheet.

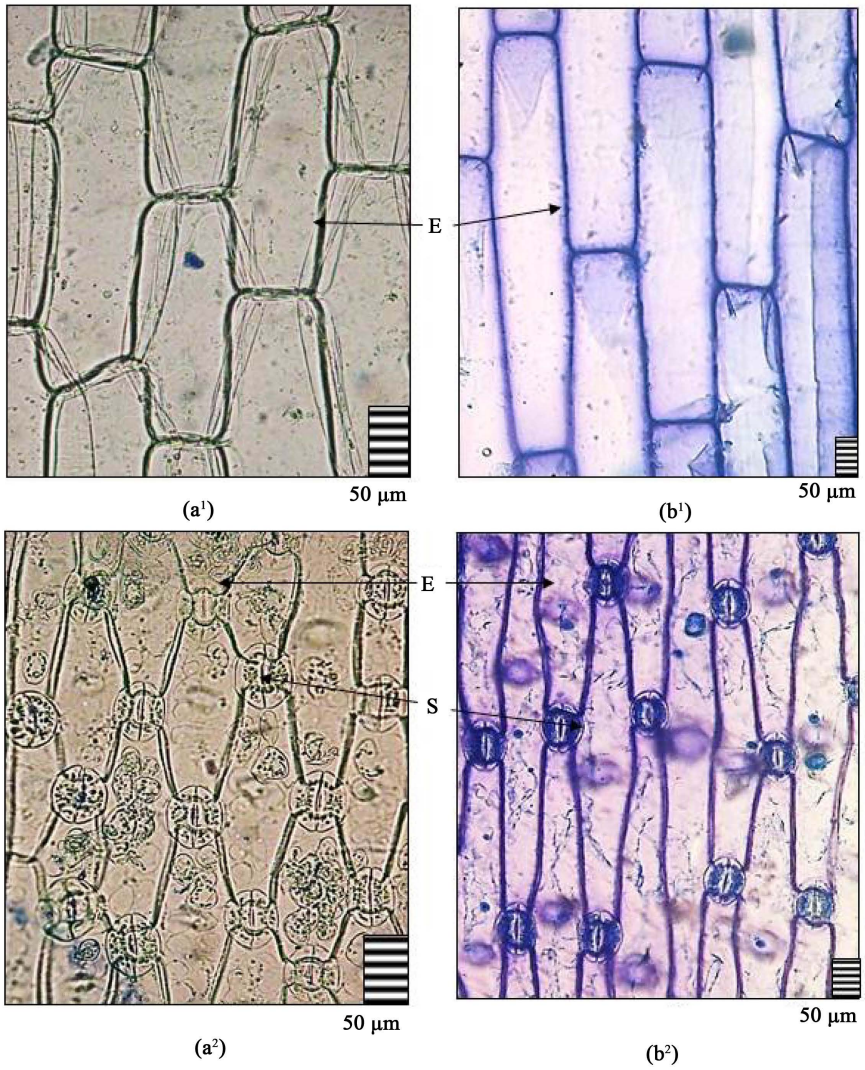

Figure 2. Anatomical structure of the epidermis of the leaf *Juno vicaria* in two different conditions of nature (a^1) - (a^2) and introduction (b^1) - (b^2): (a^1) - (b^1) adaxial epidermis; (a^2) - (b^2) abaxial epidermis. Legend: S—stomata, E—epidermis.

In a species that grows in natural conditions of the Surkhandarya region, the combination of stomatal cells (from the surface) is rounded (45.5 ± 0.41 μm in length, 40.9 ± 0.39 μm in width), the stomata belongs to the spheroid-equi-thickened type [33], which are two identical strongly round-curved cells arranged symmetrically. On the frontal plane, the thickened shells are almost uniform. Theslotisround. In a species growing under the conditions of introduction of the Tashkent Botanical Garden, the combination of stomata cells (from the surface) is oval (48.3 ± 0.40 μm in length, 63.3 ± 0.62 μm in width), the stomata is of lentil-like-thickened type, which are two identical semilunar-shaped cells are arranged symmetrically. On the frontal plane, the thickened shells are almost uniform. The slot is spindle. In a species that grows in natural conditions, the stomata are numerous (in 1 mm^2 186.5 ± 2.13), under the conditions of introduction they are few (in 1 mm^2 148.8 ± 2.27). The stomata closing cells on both sides of the leaf are almost the same length. The stomata heavily immersed (32.1 ± 0.33 μm) under the conditions of introduction, in natural conditions—poorly immersed 16.7 ± 0.17 μm, anomocytic type (**Figure 2** and **Figure 3**; **Table 1**).

Chloroplasts in the stomata guard cells can have different shapes of wall outlines, group arrangement, coarse and fine granular [33]. In a species that grows in nature, chloroplasts in the stop cells of a stomatal fine-grained form, which are scattered in the plane of stomatal cells by numerous small chloroplasts. In a species that grows under conditions of introduction, the stomatal chloroplasts of the cells (closing) are of the mid-clastic form, which are densely scattered in the plane of the stomatal cells by numerous less round-angular average sized chloroplasts (**Figure 2**).

The leaf mesophyll in a transverse section of the bendable type is represented by spongy cells on both sides of the leaf and vascular fibrous conductive bundles. The adaxial and abaxial epidermis consists of one row of cells. Adaxial epidermis cells are larger, elongated than abaxial. The thick-walled layer of the cuticle is noted in the species growing under the conditions of introduction—6.9 ± 0.05 μm, the thin-walled layer of the cuticle—under conditions of nature—5.3 ± 0.04 μm.

In *J. vicaria*, in both growth conditions, the adaxial leaf epidermis cells are large (nature—81.8 ± 1.43 μm, introduction—95.5 ± 1.33 μm), on the abaxial side of the epidermis—small (nature—21.1 ± 0.09 μm, introduction—31.8 ± 0.31 μm). Spongy parenchyma consists of large-small, chlorophyll-carrying cells. Spongy parenchyma large and unordered (5 - 6) in a species growing under the conditions of introduction (36.6 ± 0.30 μm), small and multi-row (7 - 8)—in conditions of nature (29.2 ± 0.13 μm). Between the epidermal cells and the vascular fibrous conductive bundles is angular collenchyme. In both conditions of growth in the species, the collenchyme is multi-row (nature—7 - 8, introduction—8 - 9). Large collenchyme was observed in the species under the conditions of introduction (10.3 ± 0.14 μm), small—under the conditions of nature (7.2 ± 0.09 μm) (**Figure 2**; **Table 2**).

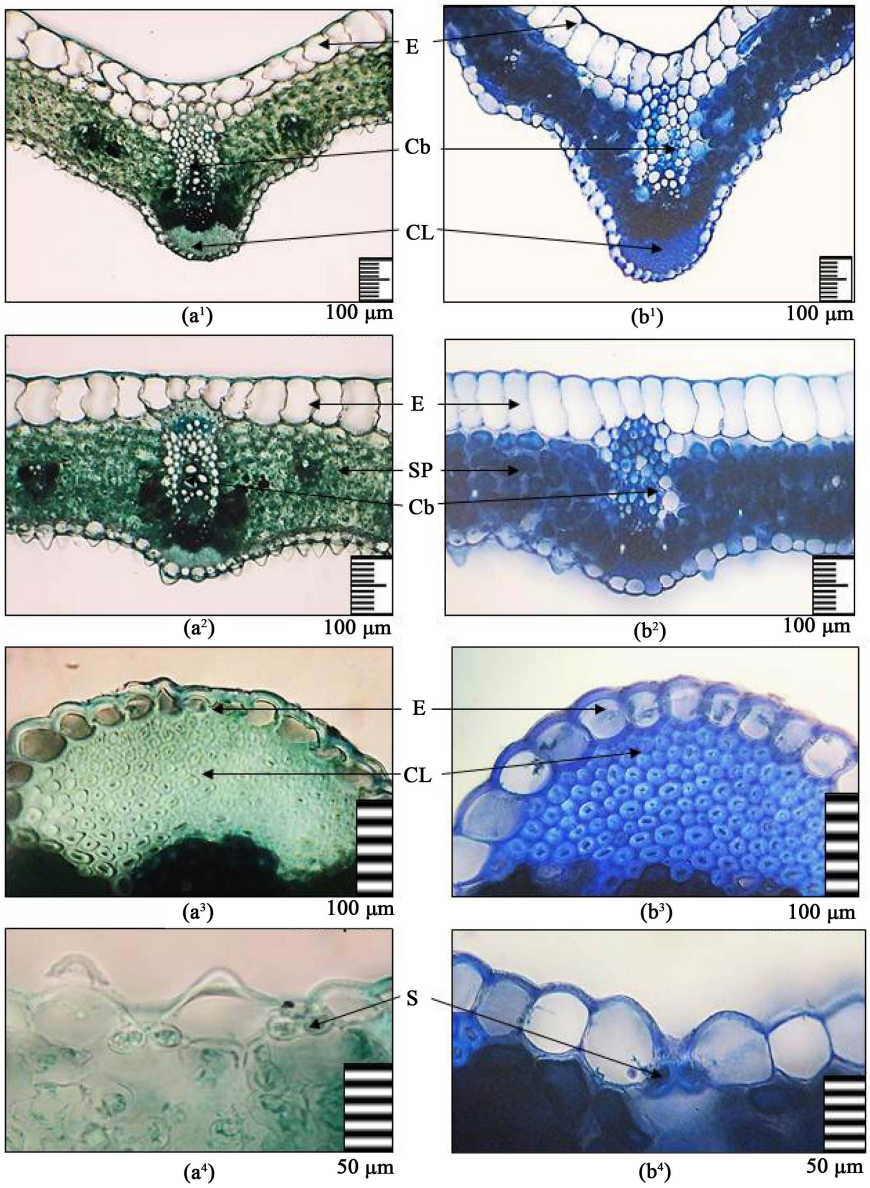

Figure 3. Anatomical structure of the leaf *Juno vicaria* in two different conditions: nature (a^1) - (a^4) and introduction (b^1) - (b^4): (a^1), (b^1) scheme; (a^2), (b^2) detail of the leaf mesophyll and conductive bundles; (a^3), (b^3) epidermis and collenchyma; (a^4), (b^4) submerged stomata and chlorophyll-bearing spongy parenchyma. Legend: SP—spongy parenchyma, CL—collenchyma, CV—conductive bundles, S—stomata, E—epidermis.

The main and lateral veins are issued on the abaxial side; there is one conducting beam. The vascular-fibrous conductive bundles are located on the periphery of the lower side of the leaf, closed collateral, numerous, consisting of phloem and xylem, with 7 - 8 large and small vessels. The largest vessels (xylem) in conducting beams were noted in a species growing under introduction conditions—22.2 ± 0.13 μm, small ones—in terms of nature—11.8 ± 0.08 μm.

The following prevailing xeromorphic and mesomorphic signs, manifested in various combinations, were determined on the basis of identifying the ratio of

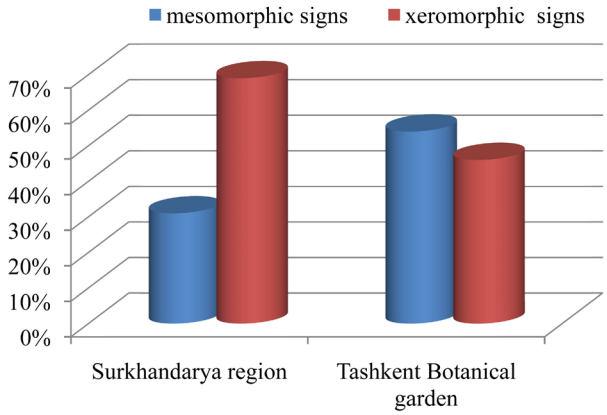

Figure 4. Ratio of adaptive mesomorphic and xeromorphic signs (n = 24) of the leaf of-*Juno vicaria* under two different conditions.

quantitative indices of the vegetative organs of *J. vicaria*: a thickened outer wall of the epidermis; small, numerous epidermal, spongy cells; numerous immersed stomata; small, multi-row sclerenchymal and collenchymal cells; small and numerous vessels in conductive beams are xeromorphic signs. Thin-walled, large, few epidermal, spongy cells; not numerous, immersed stomata; large nonmulti-row sclerenchymal and collenchymal cells; large and few vessels in conducting bundles are mesomorphic features (**Figure 4**).

In the leaf of *J. vicaria*, which grows under natural conditions, xeromorphic sings predominate; however, under the conditions of introduction more mesomorphic sings than xeromorphic ones prevail.

4. Conclusion

Thusly, the anatomical structure of the leaf of *J. vicaria* in two different environmental conditions was studied and their diagnostic features were determined. Due to their different ecological habitat, we identified adaptive mesomorphic and xeromorphic signs. A species that grows in natural habitat is dominated by more xeromorphic signs: a thickened outer wall of the epidermis; numerous epidermal, spongy cells; submerged stomata; small, multi-row sclerenchymal and collenchymal cells, which indicate a more xeromorphic and adaptability, as well as the wide distribution of this species to the natural growing conditions. In a species growing under the conditions of introduction of the Tashkent Botanical Garden, more mesomorphic features predominate: thin-walled, large, few epidermal, spongy cells; not immersed stomata; large non multi-row sclerenchymal and collenchymal cells, which show that the species is adapted to altered habitat conditions.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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