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RESEARCH ARTICLE

Leaf morphological and anatomical structure of pregenerative individuals of *Ferula tadshikorum* in *ex situ* conditions

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Abstract

For the first time, the morphological and anatomical structures of the leaves of different age individuals of the medicinal and rare species *F. tadshikorum* were studied in Tashkent Botanical Garden. Currently, natural populations of the species are in decline, and for the preservation and production of medicinal raw materials, research on the *ex situ* development conditions is needed. According to the results, adaptive responses to new habitat conditions in the anatomical structures of the vegetative organs were found. Plants of juvenile and immature development stages retained the the mesophyll's isolateral-palisade type, but differed in other quantitative and qualitative indicators of leaf tissues. Immature plants of the second year of life with more developed leaves showed modifications in the anatomical structure of the leaves, with powerfully developed vascular bundles, mechanical tissue, secretory ducts, as well as the presence of deep fibers both above the phloem of peripheral bundles, and also above the phloem of the central bundles of the petiole.

Keywords
Hypoderma, immature plant, mesophyll, palisade parenchyma, secretory duct, juvenile plant

Introduction

The genus *Ferula* L. belongs to the tribe Scandiceae Spreng. (subtribe Ferulinae Engl.) The genus includes 180-185 species of perennial monocarpic and polycarpic herbaceous plants, usually with a tall and thick stem and a strong root system. Most of them are used in traditional eastern and folk medicine (Korovin, 1947; Panahi et al., 2018; Plunkett et al., 2018). *Ferula* species are a rich source of biologically active compounds, including coumarin derivatives, sesquiterpene compounds, esters or resinous substances, flavonoids, less often saponins (Barnaulov et al., 1974; Golovina et al., 1978; Rakhmankulov et al., 1981; Zhou et al., 2017; Mohammadhosseinia et al., 2019). The use of these biologically active components with antimicrobial and insecticidal effects can provide new strategies for the development of drugs and "green" pesticides, and protect endangered plant resources (Zhou et al., 2017). The literature contains ample information on the study of the pharmacological activity of isolated biologically active compounds from various organs of *Ferula* species. Many new secondary metabolites found in *Ferula* species belong to classes of natural products with interesting biological activities from antiproliferative to anti-inflammatory and neuroprotective (Mohammadhosseinia et al., 2019). The soluble lipid fraction from the roots of *Ferula ferulaeoides* (Steud.) Korovin revealed antiviral activity against the Hepatitis B virus (Zhai et al., 2011). Volatile components of the leaves of *Ferula vesceritensis* Coss. & Durieu ex Trab. showed antibacterial activity of essential oils against gram-positive and gram-negative bacteria (Zellagui et al., 2012). Compounds of other *Ferula* species (*F. vesceritensis* Coss. & Durieu ex Trab., *F. assa-foetida* (L.) Falc., *F. gummosa* Boiss., *F. communis* L., *F. sinkiangensis* K.M. Shen.) showed cytotoxic effects, and the main mechanisms of action are associated with slowing cell growth, making them an interesting choice as an adjuvant therapy for certain diseases (Iranshahi et al., 2018). *Ferula tadshikorum* Pimenov has been used as a medicinal species in folk medicine from ancient times in treating cough, meteorism, seizures, arteriosclerosis, cataracts, nervous and mental disorders. In addition, the plant has strong anti-helminthic properties, and it is used as a spice in cooking. Both underground (milky root juice solidified in the air) and the plant's aboveground parts are used as medicinal raw materials (Sadykov, 2003; Small, 2012; Sharopov, 2018). *F. tadshikorum* is distributed in the southwestern Pamiro-Alai, and is endemic to southern Uzbekistan and southern Tajikistan. *F. tadshikorum* is not included in the Umbelliferae of the multivolume monograph "Flora of Uzbekistan" (Korovin, 1959). For the first time in 2017, when conducting resource studies of medicinal plants in southern Uzbekistan, O. Khojimatov et al. (2018) collected the herbarium material *Ferula tadshikorum*, which was identified as a new species for the flora of Uzbekistan, and its species identity was also confirmed by the monograph of the Umbelliferae family of Central Asia, Prof. M.G. Pimenov. Unsustainable human economic activity often leads to a deterioration of the vegetation cover, and as a result leads to a reduction in the number of plant populations, and possibly complete extinction. During the last two decades, due to the strong exploitation of natural populations of the species for the sake of obtaining gum resin from the roots, the condition has worsened, and the species was included in the latest edition of the Red Book of the Republic of Uzbekistan with status 3 (endangered) (Makhmudov, 2019). To preserve natural populations and prevent a further decrease in the number of *Ferula tadshikorum*, it is necessary to study the biological features of this valuable medicinal plant, by propagating seed material in *ex situ* conditions and conducting further monitoring to determine the viability of sprouted individuals. An important mechanism for the conservation of plant species is primarily the need to organize protected natural areas in natural ecosystems or create the artificial reserves, among which botanical gardens have a leading role. Botanical gardens need to pay special attention to creating plant expositions including Red-listed species and conduct educational work with the public. For the first time, the Tashkent Botanical Garden at the Institute of Botany of the Academy of Sciences of the Republic of Uzbekistan, established experimental sites to study the adaptability of *F. tadshikorum* to *ex situ* conditions. According to our data, in *ex situ* conditions, the length of the juvenile phase of *F. tadshikorum* development is much reduced. According to literary data, in natural conditions the maximum age of the species can reach 6–7 years (Rakhmonov, 2017), and in introduced conditions, some individuals in the first year of life go into the immature phase of development (Khamraeva et al., 2019). Shortening the pre-generative period of promising medicinal plants during cultivation is considered an important biological feature from an economic perspective, for the rapid production of medicinal raw materials from the underground or aboveground part. To identify the biological stability of the species in new environmental conditions, it is necessary to study the rates of ontogenesis, phenology, growth processes from which assimilating organs predefine the normal vital state of the plant. The main function of leaves is the production of organic substances, which eventually accumulate in underground organs, and a study of the structure of this organ will determine the range of its ecological adaptability to completely different environmental conditions.

The present study aimed to conduct a comparative study to detect the diagnostic signs in the morphological and anatomical structure of leaves of the individuals in the pre-generative age state *F. tadshikorum* in *ex situ* conditions.

Material and methods

F. tadshikorum grows in the middle mountain zone in the territories of the Kashkadarya and Surkhandarya regions of Uzbekistan on loess and finely ground soils and limestone, along dry river valleys and on river terraces, at altitudes of 1400–1800 m above sea level. The places of collection of seeds for subsequent cultivation under introduced conditions and a detailed description of the studied age states of *Ferula tadshikorum* were given by us in a previously published work (Khamraeva et al., 2019). The material for studying the morpho-anatomical structure of leaves of different ages was collected from experimental sites of the Tashkent Botanical Garden in 2019–2020 and preserved in 70% ethyl alcohol. The transverse sections of the leaf plate and petiole from the middle part of the leaves were stained with methylene blue and enclosed in glycerol gelatin according to the conventional method (Barykina, Chubatova, 2005). A Canon A 2300 was used to photograph the cross-sections. Statistical processing of quantitative data was carried out by G.N. Zaitsev (Zaitsev, 1990) using MS Excel. For each anatomical parameter of the leaf blade and petiole, the arithmetic mean was calculated from 30 values.

The soil and climatic conditions of the Botanical Garden are described in detail in I.V. Belolipov (1989). The garden is located in the northeastern part of Tashkent at an altitude of 480 m above sea level. The soil is an ancient rusty typical gray soil. The climate of Tashkent is sharply continental, characterized by dryness, significant daily temperature fluctuations, hot summers, dry warm autumn and moderately cold winters. The absolute minimum temperature is... – 25.8°C, the absolute maximum is... + 44.6°C. According to long-term data, the main rainfall is 380–440 mm, which falls during the autumn-winter-spring period. At the beginning of the vegetation period, watering and weeding were carried out twice in the experimental areas.

Abbreviations for figures: AbE – abaxial epidermis; AdE – adaxial epidermis; SP – spongy parenchyma; Hy – hypodermis; Cl – collenchyma; BF – bast fibers; Par – parenchyma; SD – secretory dust; Vs – vessels; Ph – phloem; Ch – chlorenchyme.

Results and discussion

Morphological structure of the leaf

We have previously studied the initial stages of the ontogenesis of *Ferula tadshikorum* in the introduction and given a detailed description of the morphological structure of the aboveground and underground parts (Khamraeva et al., 2019). According to this data, the leaf of juvenile plants of the first year of life is simple, diamond-shaped, the plate is elongated, finely serrated along the edge, 4–5 cm long and 2–2.5 cm wide, the petiole 3.0–4.0 cm long. As it grows 1–3 (5) rosette leaves form on the rosette shoot (Fig. 1a). Rosette leaves are of various lengths, from 14 to 24 cm, with the leaf plate 9–12 cm long, 4–5 cm wide, and petioles 5–12 cm long. Immature individuals of the first year of life have 4–6 simple leaves, with one triple leaf 17–25 (28) cm long, lobes obovate or wide-oval, petioles 8–16 cm long (Fig. 1b). Immature plants of the second year of life develop 1–2 scaly leaves, 7–11 cm long, which quickly wilt after the appearance of rosette leaves. Rosette leaves range from 3 to 6, of which 1–2 are simple, and 2–3 triple-dissected. In some plants more complex 5–6-lobed leaves develop (Fig. 1c, d). Triple-dissected leaves show obovate or wide oval lobes, leaf plates 15–26 cm long, petioles 12–16 cm long, while 5–6-lobed leaves have elongated oval, ovoid or broad ovate segments, leaf plates are wide-triangular in outline, up to 27 cm long, the segments 10–16 cm long, 4–6 cm wide, the primary segments with short petioles, the others sessile, with petioles up to 14 cm long.

Anatomical structure of the leaves of the juvenile plant of the first year

On the cross-section, the sheet along the entire plate is unevenly thickened, plate-like, bare (Fig. 2 a, e). Collenchyma in the central vein area is located in the ribs, on the abaxial side of 9–15 rows, adaxial – 5–6 rows, and in the lateral part - above and under the bundles, only on the abaxial side above large bundles are more multirow. The central vein area on the abaxial part shows three prominent widely spaced protrusions, and the adaxial part is closely spaced, three more or less prominent protrusions and is more thickened due to multilayer aquiferous parenchyma. In the area of large lateral veins, the leaf is biconvex (Fig. 2 d). The mesophyll consists of isolateral palisade. Under the epidermis on the abaxial side is a hypoderm single layer formed by rounded-oval cells. The palisade parenchyma on the abaxial side is 2-layered, with tabloid cells. On the adaxial side it is also 2-layered, the cells are more or less elongated (Table 1). The spongy parenchyma is 2–3-layered, with horizontally elongated homogeneous cells (Table 1).

The aquiferous parenchyma is only found in the central vein area, multilayered, consisting of up to 10 layers of various sizes thin-walled rounded-oval cells. The abaxial and adaxial epidermis are single-layered, their external walls are thickened, and both epidermises are covered with a toothed cuticle (Table 1). The central vein is represented by two vascular bundles, on the abaxial side an extensive bundle, and the adaxial part a horizontally oriented more minor (Fig. 2 b, c). Two lateral vascular bundles are located close by one above the other, and one vascular bundle is located in the side parts of the leaf in the central plane. A large main vascular bundle is represented by ten vessels, of which four are large. The vascular bundles are collateral. One secretory duct is found throughout the leaf above and below the vascular bundles, in the adaxial part with 6–7, in the area of the central vein with 10 epithelial cells, and in the abaxial part with 7–8, in the area of the central vein with 11 epithelial cells (Table 1). A large main bundle on the xylem side is accompanied by two small secretory ducts with six epithelial cells. At the edge of the leaf, the vascular bundle is surrounded by lining cells and there is one secretory duct on the abaxial part with 7–8 epithelial cells (Fig. 2 e).

Anatomical structure of the leaves of the immature plant of the first year

On the cross-section, the sheet along the entire plate is unevenly thickened, plate-shaped, abaxially pubescent with simple hairs (Fig. 3 a, b). Collenchyma in the central vein area is located in the ribs, on the abaxial side 7–18 rows, adaxial 7–10 rows, and in the lateral part - above and under the bundles, more multi-row only on the abaxial side above large bundles (Fig. 3 b, c). In the central vein area on the abaxial part there are three widely spaced, strongly prominent and two more or less prominent protrusions, and on the adaxial part with three slightly spaced more or less prominent protrusions. In the central part, the leaf is even thicker due to the multilayering of the aquifer parenchyma than in juvenile plants, and is biconvex in the area of the lateral veins. The mesophyll has the isolateral palisades. Under the epidermis on the abaxial side is a single-layer hypoderm formed by rounded oval cells. The palisade parenchyma on the abaxial side consists of 2-layered tabloid cells. On the adaxial side, it is also 2-layered, consisting of elongated cells (Table 1). The spongy parenchyma is 2–3-layered, with horizontally elongated small cells (Fig. 3 d, e; Table 1). The aquifer parenchyma is found only in the central vein area, multilayered, consisting of thin-walled round-oval cells of various sizes in up to 15 layers. The abaxial and adaxial epidermis are single-layered, their external walls thickened, and both epidermises are covered with a toothed cuticle (Table 1). The vascular bundles are of the collateral type. The central vein is represented by two vascular bundles, on the abaxial side by a large bundle, and the adaxial part is also a large double, from oppositely oriented bundles (Fig. 3 b, c). The double bundle consists of 13–15 vessels, of which seven are large; the second bundle of 8–10 vessels, of which five are large. Near the phloem there are two small secretory ducts with 5-6 epithelial cells. On two sides of the central vein one above the other there are two side bundles; on the adaxial side the bundle is inverted. One vascular bundle is arranged in the central plane of the side parts of the leaf. Large secretory ducts with up to 12 epithelial cells, and smaller ones with up to 6 epithelial cells are located above and below the lateral vascular bundles. In the central part of the leaf on the abaxial side, the secretory ducts have up to 11–13 epithelial cells, with adaxial up to 9–10 epithelial cells (Table 1). At the edge of the leaf, secretory ducts in the abaxial part with 8, and with adaxial – 6 epithelial cells (Fig. 3 e).

Anatomical structure of the leaf of an immature plant of the second year

On the cross-section, the sheet is plate-shaped, rounded-triangular in the central vein area, pubescent on the abaxial side with simple hairs (Fig. 4 a). Collenchyma in the area of central vein is located in the ribs on the abaxial side, consisting of 9–20 rows, adaxial - 7–18 rows, and in the lateral part above and under the bundles, on the abaxial side above the large bundles they are more multi-rowed (Fig. 4 b). In the central vein area, the leaf is thicker due to aquifer parenchyma than in immature individuals of the first year of vegetation, which is biconvex in the area of the lateral

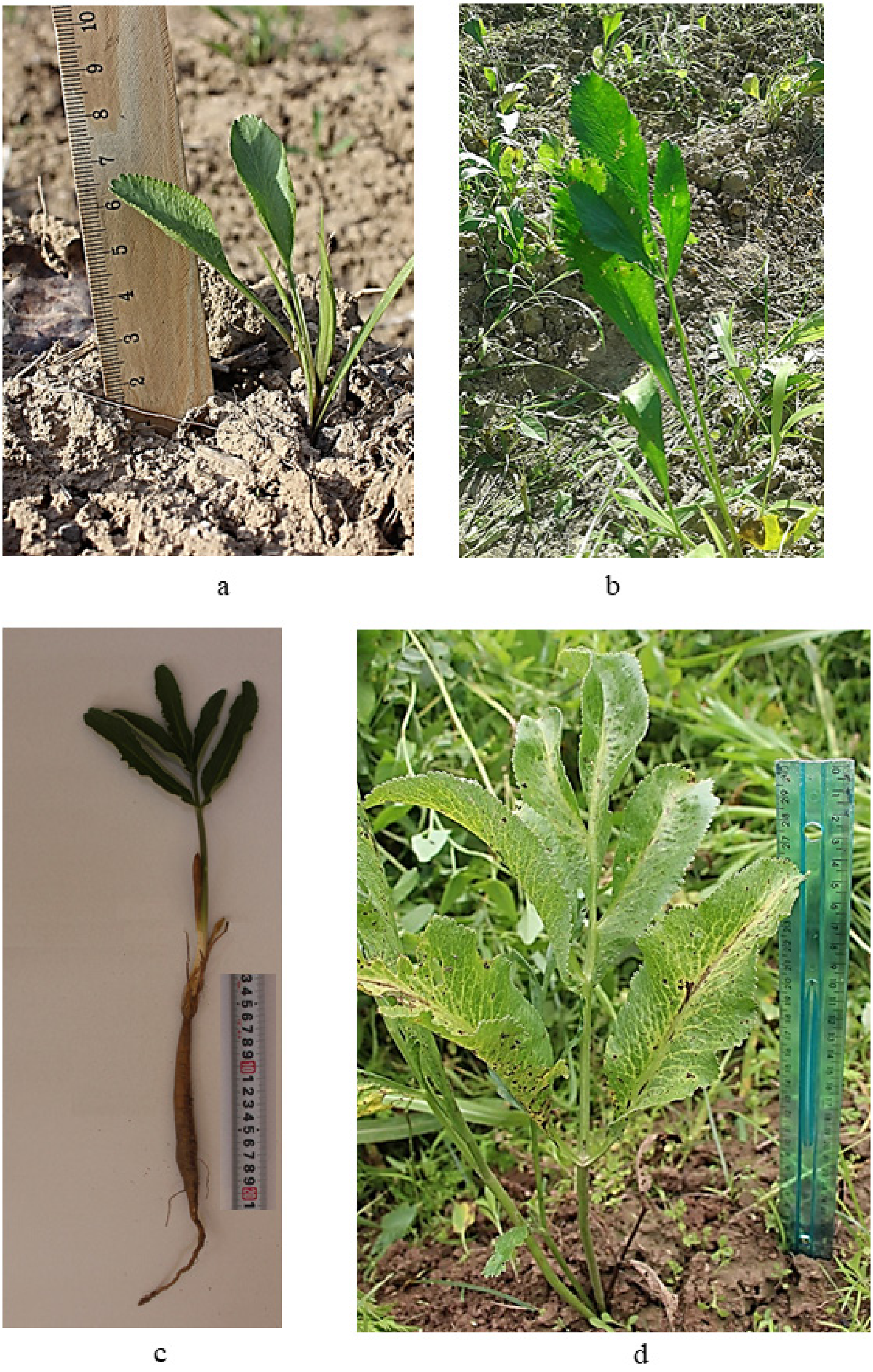

Figure 1. Different-aged plants of *Ferula tadshikorum* in *ex situ* conditions. **a** – juvenile plants of the first year (26.03.2019); **b** – immature plants of the first year (27.05.2019); **c** – immature plant of the second year (13.03.2020); **d** – immature plant of the second year (18.05.2020). Scale ruler 1 mm.

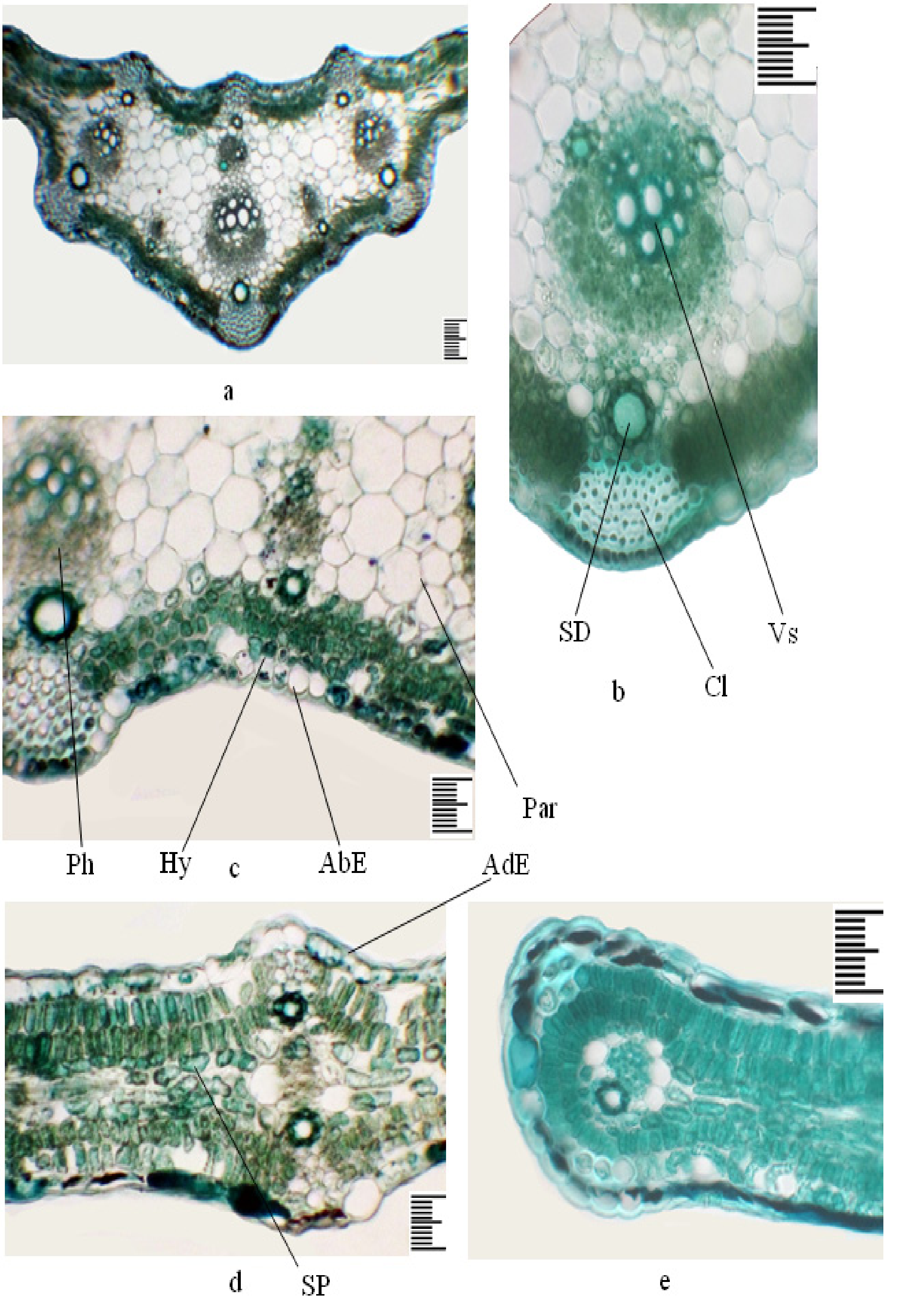

Figure 2. The anatomical structure of the leaves of a juvenile plant of the first year, *Ferula tadshikorum*. **a** – detail of the central part of the leaf; **b** – the main vascular bundle; **c** – part in the main bundle; **d** – lateral part; **e** – the edge of the leaf. Scale ruler 100 μm.

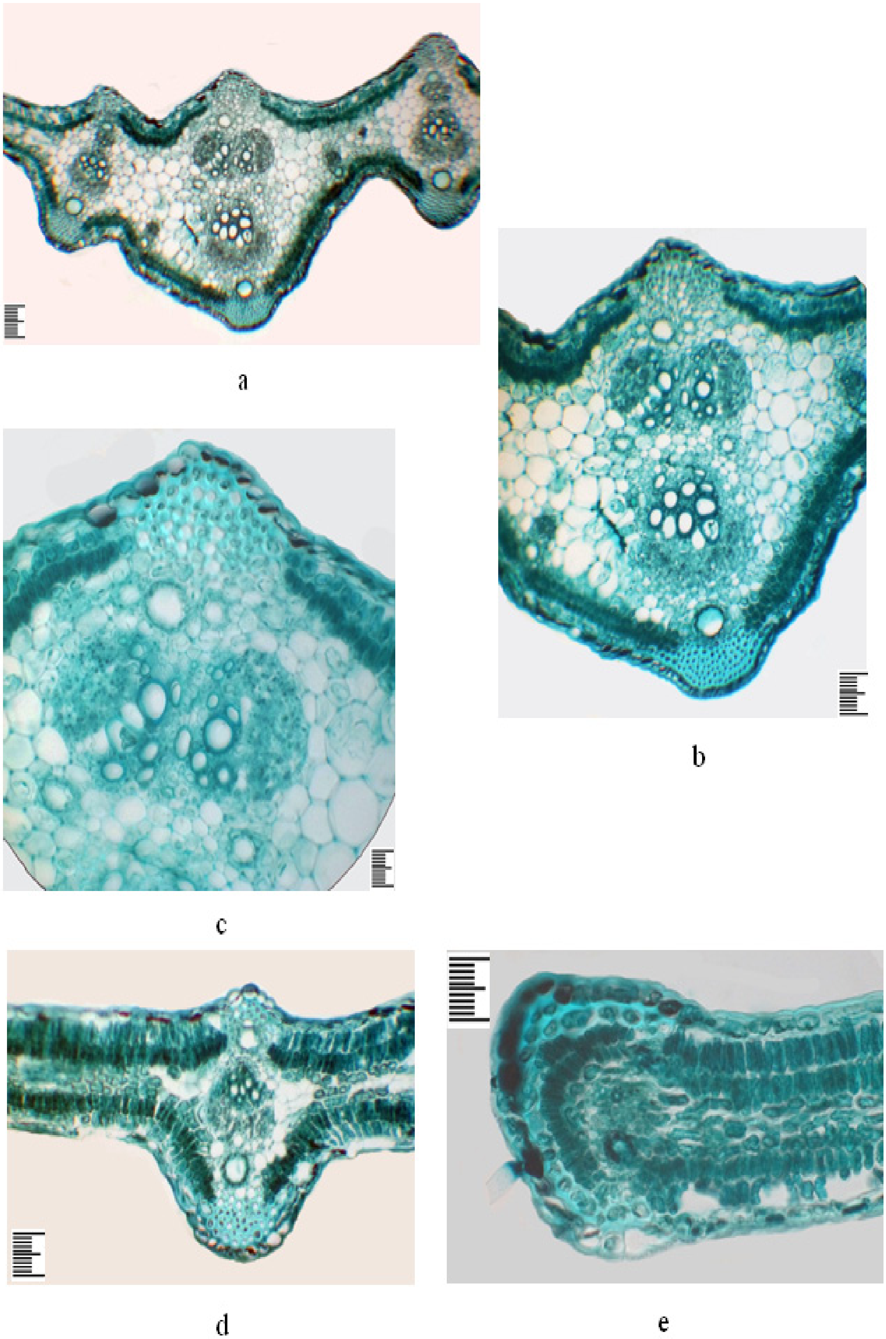

Figure 3. The anatomical structure of the leaves of an immature plant of the first year, *Ferula tadshikorum*. **a** – detail of the central part of the leaf; **b** – the main vascular bundle; **c** – part in the main bundle; **d** – lateral part; **e** – the edge of the leaf. Scale ruler 100 μm.

veins. The mesophyll is isolateral palisade. Under the epidermis in the abaxial and adaxial parts there is a single-layer hypoderm with rounded-oval cells. The palisade parenchyma in the abaxial part is 2-layered, with shortened cells; in the adaxial part it also consists of 2 layers of elongated cells (Table 1). The sponge parenchyma is 4-5 layered, with horizontally elongated small cells (Table 1). The aquifer parenchyma is found only in the central vein area, with thin-walled rounded oval cells of various sizes in up to 22 layers (Fig. 4 c, d). The abaxial and adaxial epidermis are single-layered, their external walls thickened, and both epidermises are covered with a toothed cuticle (Table 1). Vascular bundles of collateral type. The central vein is multi-bundles, represented by several large and small peripheral and one central vascular bundle. Peripheral bundles are located opposite the collenchyma strands and carry one secretory duct on the phloem side. On the phloem side, bundles are deep fibers, in large multilayer bundles (Fig. 4 b, d). There is one vascular bundle in the side parts of the leaf in the central plane (Fig. 4 e). Secretory ducts are located above and below the lateral vascular bundles, from the abaxial to 10 epithelial cells, from adaxial to 9 (Table 1). Vascular bundles in the central part of the leaf on the abaxial side have up to 10–11 epithelial cells, on adaxial up to 8–9 epithelial cells. The edge of the leaf on the abaxial side is one secretory duct with seven epithelial cells (Fig. 4 e).

Anatomical structure of the petiole of a juvenile plant of the first year

On the cross-section, the petiole is almost rounded, weakly ribbed, nominally on the adaxial side with two tubercles, strengthened by a collinear up to 10–12 rows (Fig. 5 a, c). Collenchyma strains from 7 to 12–13 rows, which alternate with 2 place three-layered chlorenchyma (Fig. 5 b). The epidermis is single-layered, covered with a cuticle, and the outer and inner walls are thickened. Under the epidermis lies a single layer of sub-epidermis whose cells contain chlorophyll grains. Around the periphery there are nine collateral-type vascular bundles, of which four are small, one is large and four are mediums. In the central part there is one vascular bundle of medium size. A large peripheral bundle carries ten large vessels and in the xylem part two secretory ducts with 6–7 epithelial cells (Table 2). The peripheral vascular bundles from the phloem part have one secretory duct with 6–10 epithelial cells, which corresponds to the size of the bundles. The main part of the petiole is represented by thin-walled rounded-oval parenchymal cells. The part phloem of the central bundle has one secretory duct with six epithelial cells.

Anatomical structure of the petiole of an immature plant of first year

On the cross-section, the petiole is rounded ovate, ribbed, nominally on the adaxial part with a small recess, collenchyma in two tubercles up to 15–18 rows (Fig. 5 d, f). The collenchyma in ribs has 8 to 20–22 rows, with large size above large bundles, smaller ones above small ones. Collenchyma strains alternate with chlorenchyma, which is a 3–4 layer. The epidermis is single-layered, covered with a cuticle, the outer and inner walls are thickened, and under it is a single-layer sub-epidermis, the cells of which contain chloroplasts. Peripheral tufts are 17, of which 11 are large, 6 smalls, all of the collateral type. There are two vascular bundles in the central part (Fig. 5 e). Above the phloem are bast fibers, above them secretory ducts with ten epithelial cells (Table 2). There are 1 or 2 secretory ducts with 6–8 epithelial cells in some large peripheral bundles of the xylem part. Above each peripheral bundle there is also one secretory duct with 10–11 epithelial cells, in small bundles up to 8. The main part of the petiole is represented by thin-walled rounded oval parenchymal cells.

Table 1. Quantitative characteristics of the leaf blade. µm

Indicators		Juvenile plants	Immature plant of first year	Immature plant of second year
The thickness of the cell outer walls	abaxial epidermis	5.13±0.14	5.55±0.06	4.41±0.14
	adaxial epidermis	5.69±0.17	5.73±0.12	4.63±0.16
Abaxial epidermis	height	17.17±0.55	13.05±0.22	20.98±1.15
	width	23.14±0.70	22.76±0.54	29.14±1.93
Adaxial epidermis	height	16.99±0.39	14.57±0.55	20.31±0.72
	width	24.31±0.83	24.52±0.48	26.45±1.08
Palisade tissue on the abaxial part	height	17.28±0.38	26.4±0.58	32.8±1.19
	width	10.36±0.22	9.62±0.26	12.16±0.45
Palisade tissue on the adaxial part	height	23.48±0.46	25.9±0.88	41.86±1.76
	width	11.9±0.19	9.56±0.23	13.46±0.46
Spongy parenchyma	thickness	63.52±1.61	42.68±0.78	64.28±2.12
Large secretory duct	height	10.44±0.28	6.42±0.11	7.63±0.27
	width	15.76±0.31	14.39±0.34	16.67±0.18
	number	10.96±0.18	10.5±0.13	9.66±0.23
Small secretory duct	height	6.66±0.15	5.28±0.12	7.05±0.21
	width	11.42±0.23	7.84±0.11	13.87±0.25
	number	8.1±0.16	7.6±0.11	9.86±0.19
Cavity diameter of ducts	large	49.82±0.91	42.93±0.75	64.7±1.22
	small	27.98±0.84	25.94±0.36	41.42±0.83

Table 2. Quantitative characteristics of the petiole. µm

Indicators	Juvenile plants	Immature plant of the first year	Immature plant of the second year
The thickness of the outer wall of the epidermis	7.7±0.14	5.4±0.16	5.4±0.20
Height of the epidermis	10.74±0.24	17.68±0.28	12.78±0.62
Cavity diameter of vessels	30.44±1.11	42.5±1.27	40±1.16
Cavity diameter of ducts	42.24±0.92	43.82±1.27	53.8±1.05
Number of ducts epithelial cells	10.16±0.18	9.23±0.17	10.23±0.34
Height of ducts epithelial cells	9.82±0.19	10.56±0.36	13.8±0.53
Width of ducts epithelial cells	16.16±0.39	16.14±0.47	18.22±0.66

Anatomical structure of the petiole of an immature plant of the second year

On the cross-section there is an ellipsoidal petiole. The epidermis is single-layered, the surface is covered with simple hairs, and the outer and inner walls are thickened. Under it is one layer of sub-epidermis with numerous chlorophyll grains. Collenchyma strains correspond to the sizes of peripheral bundles, which alternate with 4-5 layered chlorenchyma (Fig. 5 g, i). Vascular bundles are of the collateral type. Peripheral vascular bundles are represented by alternating large, medium and small numerous bundles. There are five parallel vascular bundles of various sizes in the petiole's central part, and perpendicular to the middle bundle 2 small side bundles (Fig. 5 h). Sometimes one of the central bundles is obliquely oriented concerning the rest of the central bundles. The deep fibers form caps above the phloem of the bundles; the central bundles are less multilayered. Above the peripheral bundles is one secretory duct with 10–12 epithelial cells. Numerous secretory ducts with 6–8 epithelial cells are located in the parenchymal part of the petiole. In large peripheral bundles on the xylem side there are two secretory ducts.

Growing conditions or diagnostic, morphological and anatomical features of vegetative and generative organs of some species of the genus *Ferula* have been studied (Akalın et al., 2020; Safina et al., 2014; Wang et al., 2016, Khamraeva et al., 2018; Sagyndukova, Imanbaeva, 2020). As a result of studying the anatomical structure of the above ground vegetative organs of the three ceno-populations of the rare and endemic species *Ferula iliensis* Krasn. ex Korovin found that in the stems developed mechanical and assimilation tissues located in alternating areas along the periphery directly under the epidermis, differing in volume among individuals from the different ceno-populations associated with different environmental conditions (Akhmetova, 2013). When studying the morphological features of leaves, inflorescences, fruits, and the anatomical structure of mericarp in *Ferula caspica* M. Bieb. and *Ferula szowitsiana* DC., diagnostic species-specific features of these structures were identified (Tuncay et al., 2019). For *Ferula foetida*, the diagnostic features of the leaf are the shape and structure of the epidermis cells, the presence of simple one- and multicellular trichomes, the placement of vascular bundles and the structure of secretory ducts in the leaf blade and petiole, which differ slightly in the leaves of different age individuals (Imanbaeva et al., 2015). According to S. Rakhimov and Kh. Rakhmonov (2015), in juvenile plants under natural conditions of Southern Tajikistan, in the second and third years, two scale-shaped and one diamond-shaped rosette leaves are formed annually on the rosette shoot. From the fourth to the eighth-tenth year of life in immature plants, 3-4 scaly, and 3-6 assimilating rosette leaves are formed on each annual rosette shoot, the edges of which are wavy, sometimes cut, leaves are simple, pinnately-dissected.

However, as our study in *ex situ* conditions showed, the absolute majority of juvenile individuals (95-97%) of *F. tadshikorum* go through the immature phase of development in the second year of vegetation, when the individuals have 1-2 scaly and 3-6 rosette leaves, of which 1-2 are simple and 2-3 triple-dissected, or some still pinnately-dissected with 5-6-lobes. Perhaps, in *ex situ* conditions with good moisture availability, less insolation and the absence of sharp daily fluctuations in air and soil temperature typical for mountain conditions, there is a significant reduction in the initial stages of development of the virginial period associated with new soil climatic conditions of growth. S. Rakhimov, Kh. Rakhmonov (2015) noted in natural individuals of *Ferula tadshikorum* different leaves according to the morphological structure. Studies on the morphogenesis and anatomical structure of the leaves in the closely related species *Ferula foetida* in Kyzylkum showed in juvenile and immature plants an isolateral palisade type of mesophyll, an increase in leaf pubescence on the adaxial side and an increase in the number of layers of aquifer and palisade parenchyma in the second year of vegetation (Butnik et al., 2009). According to our results, *Ferula tadshikorum* also has such signs in the structure of the leaf blade, as an isolateral palisade type of mesophyll in juvenile and immature individuals, the absence of pubescence in juvenile plants and its amplification on the adaxial side in immature individuals, and characteristic features, such as the multiplicity of the central vein, the multilayering of the aquifer parenchyma and the presence of bast fibers above the phloem of the bundles of the central vein in immature plants of the second year, the structure and location of secretory ducts. The petiole of the leaf in juvenile individuals of the first year is smaller, with a straightforward blade, and in immature plants of the second year of life it has a large, dissected leaf blade, and is characterized by the robust development of collenchyme weights and vascular bundles. Xeromorphic features in the anatomical structure of the petiole are manifested in the development of wide and narrow collenchyma strains in the ribs but differing in different age individuals in the number of rows, the thickening of the outer walls of the epidermal cells in juvenile individuals, which are most susceptible to stress-

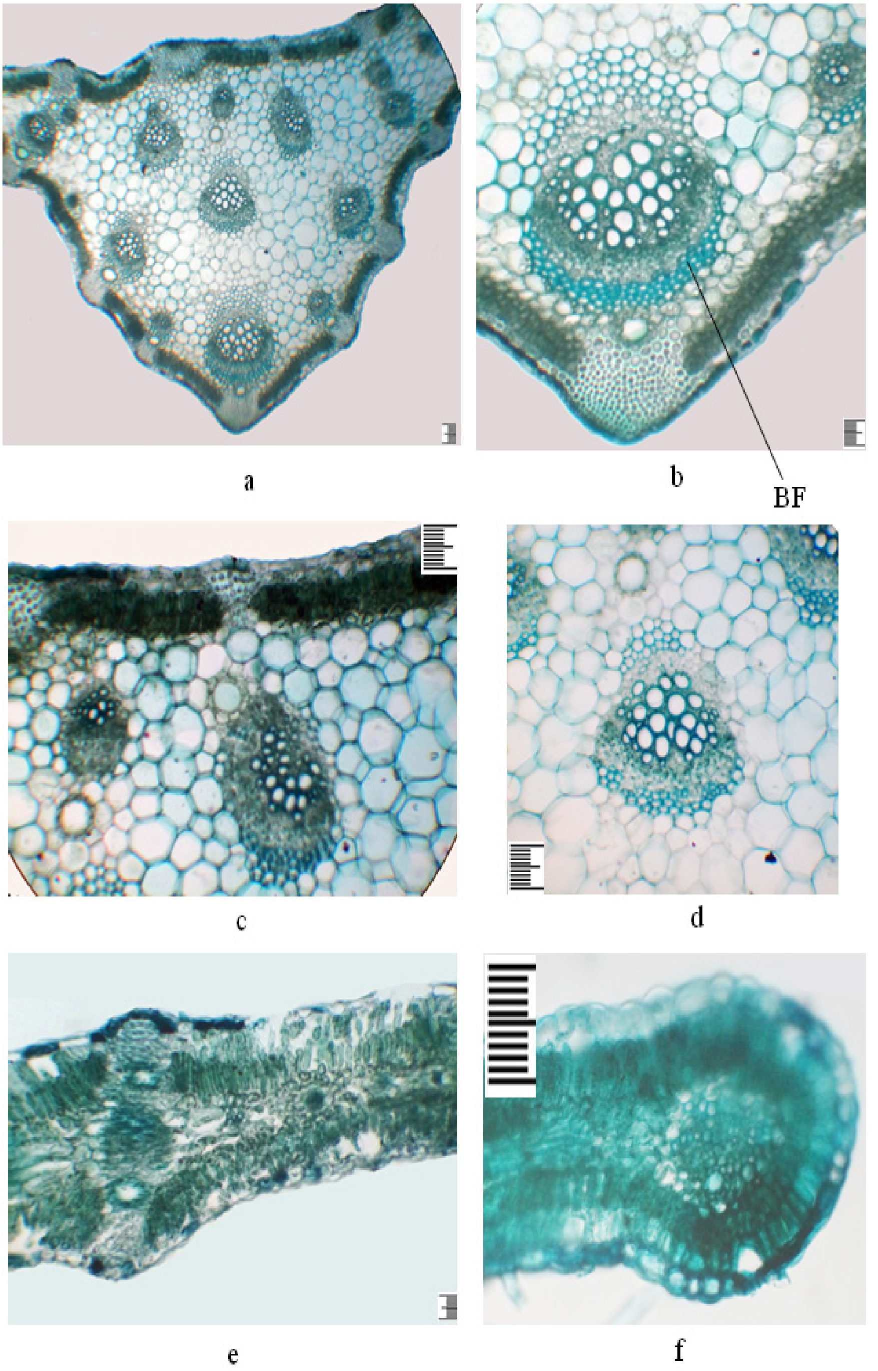

Figure 4. The anatomical structure of the leaf of an immature plant of the second year, *Ferula tadshikorum* in cultural conditions. **a** – details of the central part of the leaf; **b** – the main vascular bundle; **c** – part of the main bundle; **d** – lateral part; **e** – the edge of the leaf. Scale ruler 100 μm.

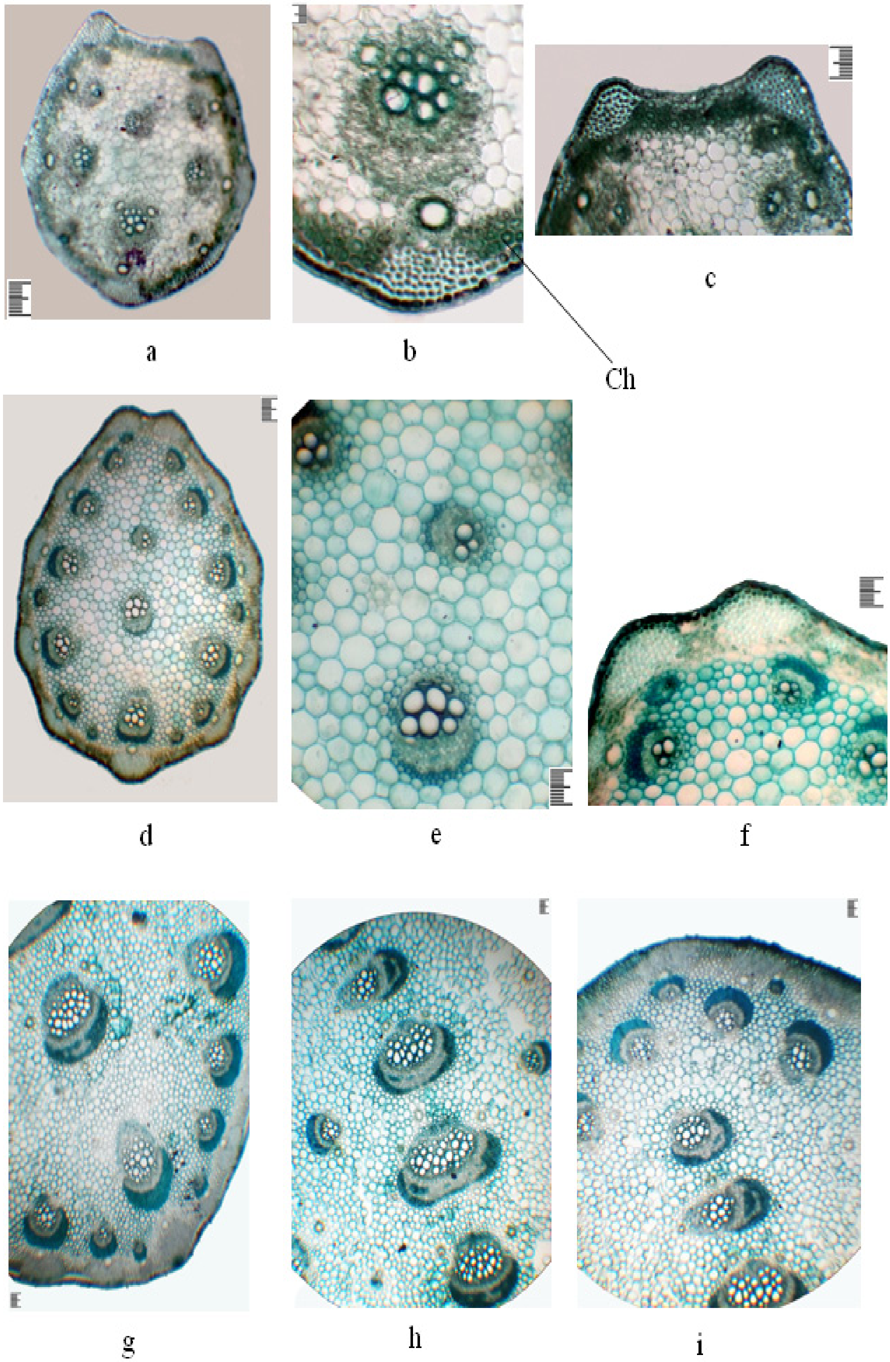

Figure 5. The anatomical structure of the petiole of *Ferula tadshikorum* plants. Juvenile plants of the first year (**a**, **b**, **c**): **a** – general view; **b** – peripheral vascular bundle; **c** – adaxial part. Immature plants of the first year (**d**, **e**, **f**): **d** – general view; **e** – central vascular bundle; **f** – adaxial part. Immature plant of the second year (**g**, **h**, **i**): **g** – detail of the abaxial part, **h** – central part, **i** – detail of the adaxial part. Scale ruler 100 µm.

ful environmental conditions, as well as in the development of additional central vascular bundles. The volume and number of central bundles increase with age. It should be especially noted that deep fibers are formed over the phloem of peripheral bundles and the phloem of central bundles in immature plants of the second year. Diagnostic features of the petiole are the shape on the cross-section of different-age individuals, which in juvenile plants are almost rounded, with two tubercles conventionally on the adaxial part, in immature plants of the first year are round ovate, with two tubercles, and in immature plants of the second year ellipsoidal. According to Kh. Rakhmonov (2017), the rosettes of shoots in species of the genus *Ferula* were formed quite early, when they developed in open habitats. This feature turned out to be adaptive in the xerophilic line of evolution of the genus *Ferula*. In this regard, the internal structure of the sheet is being rebuilt by the transition to an isolateral palisade structure, compaction of spongy tissue or reduction in the central vein area due to high air temperature differences during the day intense insolation in mountain conditions.

The results of our study showed that when plants transition from one age state to another, morphological changes in their structure, including the internal structure of the assimilated organ occur. For example, in the juvenile and immature state, the main elements of the leaf's cover, mechanical and vascular tissues are laid, which are more powerfully developed in immature plants.

References

Akalın E, Tuncay H, Olcay B, Miski M (2020) A New *Ferula* (Apiaceae) species from Southwest Anatolia: *Ferula pisidica* Akalın & Miski. Plants 9 (6): 740. https://doi.org/10.3390/plants9060740

Akhmetova AB, Mukhitdinov NM, Ydyrys A (2013) Anatomical research of vegetative organs of the *Ferula iliensis* Krasn. ex Korov., the rare and endemic species. Modern Phytomorphology 4: 223-227. [In Russian with an abstract in English] DOI: 10.5281/zenodo.161378

Barnaulov SD, Kiryalov NP, Bukreeva TV (1974). Pharmacological properties of some coumarins of the genus *Ferula* L. Plant resources 10 (2): 259-262. [In Russian]

Barykina RP, Chubatova NV (2005) Great workshop on botany. Ecological anatomy of flowering plants. KMK Scientific press Ltd, Moscow, 77 pp. [In Russian]

Belolipov IV (1989) The introduction of herbaceous plants of the natural flora of Central Asia [Introduktsiya travyanistykh rasteniy prirodnoy flory Sredney Azii]. Fan, Tashkent. [In Russian]

Butnik AA, Ashurmetov OA, Nigmanova RN, Begbaeva GF (2009) Ecological anatomy of desert plants of Central Asia 3. Herbs. Fan, Tashkent, 155 pp. [In Russian]

Golovina LA, Khasanov TX, Saidkhodjaev AI, Malikov VM, Rakhmankulov U (1978) Coumarins and esters of *Ferula microcarpa*. Chemistry of natural compounds 5: 566-570. [In Russian]

Imanbaeva AA, Sarsenbayev KN, Sagyndukova MS (2015) Anatomical structure of above- and underground organs of *Ferula foetida* (Bunge) Regel in Mangystau natural populations. Siberian ecological journal 6: 899-908. [In Russian with an abstract in English]

Iranshahi M, Rezaee R, Najaf Najafi M, Haghbin A, Kasaian J (2018). Cytotoxic activity of the genus *Ferula* (Apiaceae) and its bioactive constituents. Avicenna Journal of Phytomedicine 8 (4): 296-312.

Khamraeva DT, Beshko NYu, Abdullayeva AT, Sharipova VK (2018) Structural investigation of the secretory system of some endemic and medicinal species of Apiaceae from Uzbekistan. Iranian Journal of Botany 24 (1): 53-64.

Khamraeva DT, Khojimatov OK, Uralov AI (2019) Growth and development of *Ferula tadshikorum* Pimenov in culture. Acta Biologica Sibirica 5 (3): 172 – 177. [In Russian with an abstract in English] https://doi.org/10.14258/abs.v5.i3.6588

Khojimatov O, Maltsev I, Turginov O (2018) On the issue of stocks of the medicinal plant *Ferula tadshikorum* in Uzbekistan. Ecological Bulletin of Uzbekistan 1 (201): 24-26. [In Russian]

Korovin EP (1947) Genus *Ferula* (Tourn.) L. monographia illustrata. Academiae Scientiarum UzRSS, 91 pp. [In Russian]

Korovin EP (1959) Flora of Uzbekistan 4. Fan, Tashkent, 257-470. [in Russian]

Makhmudov AV (2019) *Ferula tadshikorum* Pimenov. In: The Red Data Book of the Republic of Uzbekistan. Plants 2: 95-96. [In Russian]

Mohammadhosseinia M, Vendittib A, Sarker S, Naharc L, Akbarzadeh A (2019) The genus *Ferula*: Ethnobotany, phytochemistry and bioactivities – A review. Industrial Crops & Products 129: 350-394.

Panahi M, Banasiak Ł, Piwczyński M, Puchałka R, Kanani MR, Oskolski AA, Modnicki D, Miłobędzka A, Spalik K (2018) Taxonomy of the traditional medicinal plant genus *Ferula* (Apiaceae) is confounded by incongruence between nuclear rDNA and plastid DNA. Botanical Journal of the Linnean Society 188 (2): 173-189. https://doi.org/10.1093/botlinnean/boy055

Plunkett GM, Pimenov MG, Reduron JP, Kljuykov EV, Van Wyk BE, Ostroumova TA, Henwood MJ, Tilney PM, Spalik K, Watson MF, Lee BY, Pu FD, Webb CJ, Hart JM, Mitchell AD, Muckensturm B (2018) Apiaceae. In: Kadereit J, Bittrich V (eds) Flowering Plants. Eudicots. The Families and Genera of Vascular Plants 15: 1-198. https://doi.org/10.1007/978-3-319-93605-5_2

Rakhimov S, Rakhmonov Kh (2015) Ontogenesis of the monocarpic shoot *Ferula tadshikorum* M. Pimen. Bulletin of the Academy of Sciences of the Republic of Tajikistan 1 (189): 7-11.

Rakhmankulov U, Melibaev S, Saidxodjaev AI (1981). Central Asian species of the genus *Ferula* L. – sources of sesquiterpene derivatives. Biological features and distribution of promising medicinal plants. Fan, Tashkent, 138-153. [In Russian]

Rakhmonov KhS (2017) Biology and resources of *Ferula tadshikorum* M. Pimen. in the South of Tajikistan. Dissertation for the degree of Candidate of Agricultural Sciences. Dushanbe, 179 pp. [In Russian]

Sadykov YuD (2003) Biologically active substances of wild medicinal plants in Tajikistan: Content, biosynthesis and practical use. Dissertation for the degree of Doctor of Biological Sciences. Dushanbe, 325 pp. [In Russian]

Safina K, Ostroumova T, Pimenov M (2014) Carpology of the species of *Ferula* subgen. *Merwia* (Umbelliferae–Apioideae) and some taxonomic implications. Nordic Journal of Botany, 33: 140-150. https://doi.org/10.1111/j.1756-1051.2013.00315.x

Sagyndukova MS, Imanbaeva AA (2020) Study of the anatomical structure of *Ferula foetida* of different age states and origin. Bulletin of the Karaganda University 1 (97), 73-78. [In Russian with an abstract in English]

Sharopov FS, Khalifaev PD, Satyal P, Sun Y, Safomuddin A, Musozoda S, Wink M, Setze WN (2019) The chemical composition and biological activity of the essential oil from the underground parts of *Ferula tadshikorum* (Apiaceae). Records of Natural Products 13(1): 18-23. http://doi.org/10.25135/rnp.65.18.02.089

Small E (2012) Top 100 exotic food plants. CRC Press, New York, 708 pp.

Tuncay H, Olcay B, Uruşak E (2019) Comparative morphology and fruit anatomy of *Ferula szowitsiana* DC. and *Ferula caspica* M.Bieb. Journal of Research Pharmacy 23(3): 577-583. https://doi.org/10.12991/jrp.2019.165

Wang X, Liu M, Ru J, Wang J, Wang Yu (2016) Morphological and anatomical characteristics of the fruit of Chinese *Ferula* and related taxa in Apiaceae. Acta Prataculturae Sinica 25 (6): 81-93. DOI: 10.11686/cyxb2015458

Zaitsev GN (1990) Mathematics in experimental botany. Nauka, Moscow, 296 pp. [In Russian]

Zellagui A, Gherraf N & Rhouati S (2012) Chemical composition and antibacterial activity of the essential oils of *Ferula vesceritensis* Coss et Dur. leaves, endemic in Algeria. Organic and Medicinal Chemistry Letters 2: 31. https://doi.org/10.1186/2191-2858-2-31

Zhai LL, Liu T, Xie HQ, Xie YH, Mu Q (2012) Inhibition effects on HBV replication by hydrophobic extracts from *Ferula ferulaeoides* (Steud.) Korov. Journal of Medicinal Plants Research 6 (8): 1486-1488. https://doi.org/10.5897/JMPR11.553

Zhou Yu, Xin F, Zhang G, Qu H, Yang D, Han X (2017) Recent Advances on Bioactive Constituents in *Ferula*. Drug development research 78 (7): 321–331. https://doi.org/10.1002/ddr.21402