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Comparative study of root, stem, and leaf anatomy of young Sogdian ash trees (*Fraxinus sogdiana* Bunge) growing in river valleys of the Sharyn State National Park

Abstract. The study of endangered relict woody plants is a key component of biodiversity conservation strategies. In this regard, the purpose of the study was to compare anatomical features of the vegetative organs of relict Sogdian ash (*Fraxinus sogdiana* Bunge) growing in river valleys of the Sharyn State National Park in the Almaty region. In 2020-2021 young individuals of Sogdian ash were sampled, in the valleys of the Sharyn and Temirlik rivers to study, their root, stem, and leaf anatomical features for the first time. The lower and upper epidermis of *F. sogdiana* leaves were thicker in individuals growing at the Sharyn site than at the Temirlik site. In contrast to that, the thickness of the mesophyll was thicker in leaves from the Temirlik site. The cross-sectional area of the conductive bundles of plants were larger in trees from the Sharyn site. The morphometric parameters of the stem also had different results. The diameter of the roots were twice as large in Sogdian ash trees growing at the Sharyn site, likely due to the growing conditions, since the soil of the Sharyn River valley is meadow, stony and pebbly, compared to the Temirlik River, which is a deposit of red clay mixed with crushed stone and rock remnants. Since Sogdian ash is drought tolerant dry air well, due to its resistance to heat and soil salinity, it can be recommended in order to preserve biodiversity, for landscaping, and for improving ecosystem conditions.

Key words: Sharyn National Park, Sogdian ash (*Fraxinus sogdiana* Bunge), relict, anatomy, morphometry.

Introduction

The ash tree genus *Fraxinus* is one of the largest in the family of *Oleaceae*. This family contains 27 genera and 687 accepted species names (WcSP, 2017-World Checklist of Selected Plant Families). The genus *Fraxinus* contains 53 species. It also contains hybrids and many intraspecific names. Plants of this genus grow as deciduous trees and shrubs. They are mainly found in temperate forests and in forests in the northern hemisphere. Several species can be found in tropical locations in Central America, India and parts of Indochina. Two species are found in North Africa (WcSP, 2017). Fraxinus species are widely used, such as for landscaping, in the wood industry, and also as a food source for livestock. Although *Fraxinus* species are often wide distribution range, some species at risk in the wild due to small geographic ranges. Even those that currently have an extensive range are susceptible to invasive pests and diseases, such as the emerald ash, *Agrilus planipennis*, in north America, and the fungal pathogen *Hymenoscyphus fraxineus* in Europe that causes desiccation [1].

The Red List of Fraxinus well illustrates tree species disappearing around the world at an alarming rate. These threats may cause rapid population declines in the future. Therefore, it is necessary to evaluate the genus *Fraxinus* in order to preserve the endangered species of these valuable trees.

In order to ensure the sustainable development of arid oasis cities, close attention must be paid to the use of limited water resources. Since urban vegetation uses an important amount user of water, urban greening activities must be adapted to local environmental conditions. Two introduced urban green tree species (*F. sogdiana* Bunge and *Platanus acerifolia* Willd.) were selected by Chinese scientists in China. These species developed certain strategies to adapt to arid environment conditions and thus consume less water. *F. sogdiana* has a relatively high drought tolerance and compared with *P.acerifolia*, which consumes less water, so it is suggested when choosing urban landscaping tree species [2]. The inclusion of ash species in the urban catalog of tree species for the description and identification of harmful substances caused by pollution makes it possible to identify the environmental crisis in cities and megacities in the short and medium term [3-5].

F. sogdiana is a vulnerable species, therefore it is timely and an important first step to prioritize tree species for conservation efforts to reverse the declining trend of species worldwide. To assess the impact of ash shrinkage from *Chalara fraxinea*, which has already been used to represent the density and size trajectories of pure single – aged stands of beech, oak and douglas fir, it has proven its ability to display the size density trajectories for a new species-ash. The widespread drying of the ash caused deviations from the expected particle density trajectories. These deviations can be used to determine the additional mortality rate due to *Hymenoscyphus fraxineus* infection in pure single-aged ash trees. New ideas for studying the inexpensive and highly effective modified adsorbent biochar as a new biochar to eliminate tetracycline from water [6-9]. The factor responsible for the higher infestation of seedlings, the low water table affected the density of natural renewal of ash trees. The direct and indirect relationship between the properties of the soil and the natural regeneration of ash is revealed. The most important factors with the density of ash regeneration correlate the proportion of seedlings, soil pH and CaCO3 content together with soil moisture. This is important for predicting reforestation and the ability to recover from disturbances caused by H. *fraxineus* [10].

Fraxinus species are of great interest for forest resilience, as they can survive fire or drought by activating dormant vegetative buds to resume growth. Understanding what factors control the early growth and survival of these species is essential for developing strategies to increase the resilience of plantings [11]. The anatomical parameters of the leaves of various *Fraxinus* correlate with the physiological parameters of the leaves of other woody plants. Absorbent and transport thin roots with a diameter of less than 2 mm differ greatly in anatomy, morphology and physiology, as well as in their response to environmental changes [12-17].

The main botanical feature of the territory of the State National Natural Park «Sharyn» in the Almaty region is the ash forest dacha, which was declared the natural monument by the Resolution of the Council of Ministers of the Kazakh SSR from March 19, 1964. The nature monument was created to protect the floodplain forest formed by a relic of the Paleogeon period-the Sogdian ash (river-loving). It is located in the Sarytogay tract in the floodplain of the Sharyn River and is currently part of the SHGNPP [18].

The scientist G. E. Grum-Grzhimailo, 1896 notes in his "Description of the way to Western China" that the inhabitants of the Turfan valley plant elm, tut, ash and other trees for shading in the villages and points out that ash is locally called "Sharyn" [19].

In 1944, B. A. Bykov states in his article "Relict Chagan forest in the Charyn River valley" that this forest of moisture-loving ash trees (*Fraxinus potamophila* Herd) should be preserved as an amazing natural monument. At that time, a whole wood processing plant was working on the basis of these forests, so he was afraid that the merciless cutting of ash trees along the Sharyn River would quickly lead to the complete destruction of ash groves [20].

Sogdian ash (*F. sogdiana* Bunge) is a rare, relict species with a disjunctive habitat. According to the literature, in 1926 the natural area of the ash grove was 1100 hectares in the SNNP "Sharyn", in 1943 it decreased to 410 ha, and in 1981 increased to 812 ha. Currently, the area occupied by *F. sogdiana* Bunge is 5,014 thousand ha.

The rarity of the plant is due to the stenotopic nature of the species, high quality wood and intensive economic use. It is included in the Red Book of the Republic of Kazakhstan and in the IUCN Red List (International List of Rare and Endangered Species, 1964) with the category "Near Th reatened" – a condition close to threatened. The largest natural population is located in the Sarytogay tract in the Sharyn National Park.

To preserve biodiversity, Sogdian ash was introduced in the valleys of the Ayak-Sungy and Boroldai rivers in the south of Kazakhstan. Therefore, in the modern literature, much attention is paid to the introduction of this species. On the territory of the Syrdarya-Turkestan State Regional National Park, F. sogdiana forms plantations on an area of 536 hectares. Sogdian ash (*F. sogdiana* Bunge) is a tree 10-15 m tall, elongated-ovate or spherical crown, reddish-brown cracked bark. *F. sogdiana* lives in floodplains and foothills with close occurrence of groundwater [21; 22].

The construction of large dams and the creation of reservoirs lead to changes in the natural course of the hydrological regime and endanger the existence of unique floodplain forests of Sogdian ash (*Fraxinus sogdiana*), listed in the IUCN International Red List [23]. At present, the risk of degradation of the Ash Grove is increasing. The main danger is the construction of the Moinak hydroelectric power station, which violates the natural hydrological regime of the river and negatively affects the preservation of the Ash Grove. Since Sogdian ash is a moisture-loving species, a slight decrease in the amount of water or a decrease in the level of groundwater can negatively affect its growth and natural restoration.

Necessary protection measures include: improvement of the hydrological regime of the Sharyn river to improve natural and artificial reforestation [24].

The anatomical structure of Sogdian ash (*F. sogdiana* Bunge) has not been previously studied in Kazakhstan. The aim of this study was therefore, to investigate root, stem, and leaf anatomy of the vegetative organs of the Sogdian ash.

Materials and methods

Morphology. In its natural distribution range, Sogdian ash can reach up to 25-30 m in height. Branches are reddish-brown or brown, almost dark brown, and short pubescent. Leaves are up to 20 cm long, opposite, on fruiting twigs of three in whorls, unpaired, rarely paired, 3-6 paired, pairs of leaflets strongly spaced one from the other, leaves are ovate, lanceolate or narrowly lanceolate, 2-6 cm long, 1b5-3 cm wide, glabrous from below, top slightly hairy at the base, short or long pointed at the top, sharp-toothed at the edges, on short petioles or almost sessile; inflorescence racemose, short racemes, up to 5 cm long, emerge from the axils of last year's leaves; flowers 2-3 in whorls, without calyx and corolla; stamens 2; winglet elongated-elliptical or lanceolate, 3-3. 5 cm long at the base slightly twisted, pointed or blunted; nut cylindrical, shorter than the length of the winglet or equal to half of it. Flowers VI, fruits VII [24,25].

The object. F. sogdiana Bunge, samples of plant raw materials were collected during July, in 2020, the foothills located within the Ili intermountain basin, Almaty region, samples were collected on the floodplains of the Temirlik River and on the floodplains of the Sharyn River. Anatomical studies were carried out on the vegetative organs (root, stem, leaf) of *F. sogdiana* Bunge, in 2021.

Ash grove is located on the floodplains of Sharyn River. In the formed phytocenosis of Sharyn, young ash individuals were found in herbaceous plants-cereals communities, with willow gatherings, where dominated Jungar willow (Figure 2).

Figure 1 – *F. sogdiana* Bunge

Figure 2 – Floodplain of the Sharyn River

Figure 3 – Floodplain of the Temirlik river

On the floodplains of the Temirlik River, there are individual single ash trees that form small populations. Composition of the phytocenosis: elagnus-salix with *F. sogdiana*. Young trees of *F. sogdiana* were collected for analysis from this community, Figure 3. The GPS coordinates of *F. sogdiana* in the floodplains of 2 rivers are given in Table 1.

Anatomy and morphometry. An anatomical study was performed on the fixed material. Cross-sections of roots, stems and leaves were prepared manually and with the help of a microtome on the TOS-2 freezing device. The object was covered with a cover glass, viewed from both sides under a microscope, first at a small (x70, x100), then at a large (x200) magnification. The sections were examined in glycerol.

For quantitative analysis, the morphometric parameters were measured using the MOV-1-15 eyepiece micrometer (with a lens x 9, magnification x 10.7). For the study, the average morphological data of 15 sogda shagany trees taken from the Sharyn River Valley were 32-39 CM, and 15 young individuals from the Temirlik River Valley were taken, with an average length of 37-41 CM. Samples of 15 roots, 15 leaves, and 15 stems were taken from individuals from the Sharyn and Temirlik river valleys.

Micrographs of the anatomical sections were taken on a MC 300 microscope (Micros, Austria) with a CAM V400/1.3 M video camera (JProbe, Japan). The microscopic study was carried out on the basis of the laboratory of "Plant Anatomy and Morphology" of al-Farabi Kazakh National University. Microscopic studies were performed to determine the morphological and anatomical features of the roots, stems and leaves. When describing the roots, the following features are of particular importance: on a cross-section, at a small magnification (10x), it is necessary to distinguish the primary bark, which most often occupies a large part of the root section, and a relatively narrow central cylinder. Their general outline, shape and structure of cells, as well as the distribution of xylem and phloem elements are described.

Table 1 – GPS coordinates of the studied plant species

SNNP "Sharyn"		
Geographical location	Ili intermountain basin	
Date	01.07.2020г	02.07.2020г
Time	12.24	11.30
North latitude coordinates	43 º 33' 27.1"	43³º 21' 31.4"
Coordinates of east longitude	79 º 17' 58.3"	79 º 09' 56.5"
Height	706	955
Landscape	Floodplain of the Sharyn River Sharyn	Floodplain of the Temirlik river
Water regime	Sharyn river	Temirlik river

Sections of the roots were made in their basal part along their entire length every 2-3 cm. In the manufacture and description of the preparations, the methods generally accepted in plant anatomy were used [26-28]. Sections made from the middle part of the stem, leaf and root samples were studied and 100 preparations were prepared from each organ.

When analyzing the data obtained, statistical processing was used, using the *Microsoft Office* software package. The description of external features is made in accordance with the requirements of State Pharmacopoeia XI [29-30].

Results and discussion

Anatomical structure of the root of F. sogdiana. Cross-sections of the roots of F. sogdiana Bunge from the Temirlik site (Figure 4, 70x), shows peeling layers of the periderm (1) (a dark green layer of cells), forming rows of peripheral cells. Next, the cells of the parenchyma of the primary cortex are arranged in more or less 2-3 concentric layers (2). The parenchyma cells are rounded with slightly thickened walls without intercellular cells. The secondary phloem (3) is represented either by a continuous concentric cell layer or by intervals areas around the cambium cell layer (4). The secondary phloem consists mainly of the ligamentous and radial parenchyma; there are relatively few sieve tubes in it. The cambial layer is represented by tightly closed cells, which borders on the central cylinder. The pericycle is represented by a single layer of cells. The secondary xylem (5) is represented by 5-7 radial chains of narrow-light small and medium vessels (7) converging to the center of the central cylinder. The cross-section area of xylem vessels was in average *0.035 x 10-3mm*^{2}. In the center, the cells of the core parenchyma are localized (6). The primary wood (xylem) is usually pentarchic (five-ray).

When examining a cross-section of the root of *F. sogdiana* from the Sharyn site (Figure 5, at 70x magnification), a similar structure was noted. The difference in the severity of the core rays, which were more numerous in this sample, and they are observed to a greater extent (8). Also, a looser structure of all root tissues is noted. In the process of growth, a secondary thickening of the root takes place, which was considered on anatomical sections. Minor differences were found in the morphometric parameters of the root structure of *F. sogdiana*. The morphometric parameters of the roots are presented in Table 2.

Based on the results of the Table 2, it should be noted that the primary bark of the root of *F. sogdiana* from the area of the Sharyn River is thicker (8,603±1,6 mkm) than in plants from the area of the Temirlik River (3,651±1,9 mkm). The difference was twice as large in ash trees from the Sharyn River area, which is probably due to depending on the composition of the soil. The opposite pattern is observed regarding the diameter of the central cylinder. In plants from the Sharyn River area-22,132 microns and 24,189 microns from the Temirlik River area, the central cylinder is slightly larger. With respect to the area of xylem vessels and the thickness of the secondary phloem layer, the results obtained for the average values are relatively the same.

Anatomical and morphological structure of the stem of F. sogdiana. The anatomical and morphological structure of the stems of *F. sogdiana* (Figure 6) from the *Temirlik* site differs significantly from the structure of the stem of *F. sogdiana* (Figure 7) from the *Sharyn* site. Stem cross-section of *F. sogdiana* of the *Temirlik site* (Figure 6) shows a rounded outline. On the surface is the periderm (1), with a layer of cuticle. The periderm layer is followed by the primary cortex (2), with cells of a rounded and rounded – oblong shape. The elements of the phloem (bast) (3) are arranged in several layers. The bast consists of lignified thick-walled cells separated by parenchyma. The cambial layer (4), bordering the secondary cortex, is represented by several rows of tabular cells. Inside of the cambium is secondary wood with growth rings. The central cylinder has a well-developed mechanical tissue-the sclerenchyma (5). Cells with thick cell walls that lie in several layers (4-5). The xylem vessels (6) are quite small, tracheids are rare, wood fibers are thick-walled. Along the periphery of the stem, the wood cells are located diffusely throughout the annual ring and form a lining around the vessels (such a parenchyma is called vasicentric). The inner part of the stem is represented by large, loosely arranged parenchymal core cells (7).

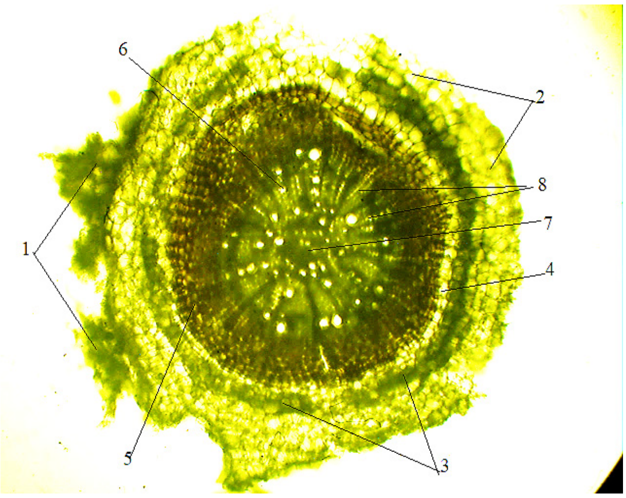

Figure 4 – Anatomical structure of *F. sogdiana* (Temirlik river) root (x70). Note: 1-periderm, 2-primary cortex, 3-secondary phloem, 4-cambium, 5-secondary xylem, 6 – xylem vessels, 7-core parenchyma

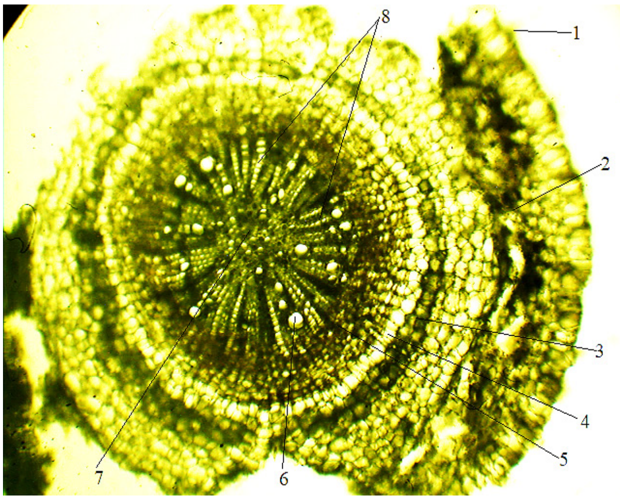

Figure 5 – Anatomical structure of *F. sogdiana* (Sharyn river) root (x70). Note: 1-periderm, 2-primary cortex, 3-secondary phloem, 4-cambium, 5-secondary xylem, 6-xylem vessels, 7-core parenchyma, 8-core rays

Table 2 – Morphometric parameters of *F. sogdiana* roots

Area	Thickness of the primary cortex, mkm	Diameter of the central cylinder, mkm	Secondary phloem layer thickness, mkm	Cross sectional area of xylem vessels, x10^{-3}mm^2
river Temirlik average value	3,651±1,9	24,189	1,009±0,2	0,007±0,02
river Sharyn average value	8,603±1,6	22,132	1,009±0,002	0,0416±0,008

Stem cross-section of *F. Sogdiana*, from the Sharyn site (Figure 7) has rounded faces, including 3-4. The periderm (1) is much more pronounced, consists of phellogen and its derivatives – phelloderm (deposited inside) and phellem, or cork (secondary integumentary tissue, deposited outside). Numerous epidermal formations – simple hairs-are marked along the entire surface of the stem (2). In the parenchyma of the primary cortex, a layer of collenchyma is marked, represented by 2-3 layers of cells. Among the cells of the parenchyma (5), closer to the cambium is the secondary phloem (6), which consists of rare bast fibers and groups of stony cells. The broadest elements of the soft bast are sieve-shaped tubes with horizontal sieve-shaped plates, which are clearly visible on the cross-section. The cambial zone (7) is similar to that of the previous sample. The secondary xylem (8) is represented by vessels that are located among numerous cells of the sclerenchyma (9). The vessels of the xylem (10) are quite small, and there are also larger ones. The core is represented by a pronounced large-cell storage parenchyma, which occupies two to three times more area than in the previous sample from the Temirlik river area.

The morphometric parameters of the stems are presented in Table 3.

Based on the results of Table 3, it should be noted that the thickness of the periderm in plants from the area of the Sharyn river is 1.066 µm, and the area of the Temirlik river is 0.061 µm. It differs significantly, whereas for the thickness of the primary cortex plants from the Sharyn river area show thickness of 1.094 µm, while plants from the Temirlik river area show a thickness of -*2.225* µm. The diameter of the central cylinder in plants of the Sharyn river is twice as large (84,801 microns) as in plants of the Temirlik river (41,096 microns). The thickness of the sclerenchymal layer in the central cylinder differs slightly. These anatomical features of the stem organization indicate the peculiarities of the growth of this species in various conditions.

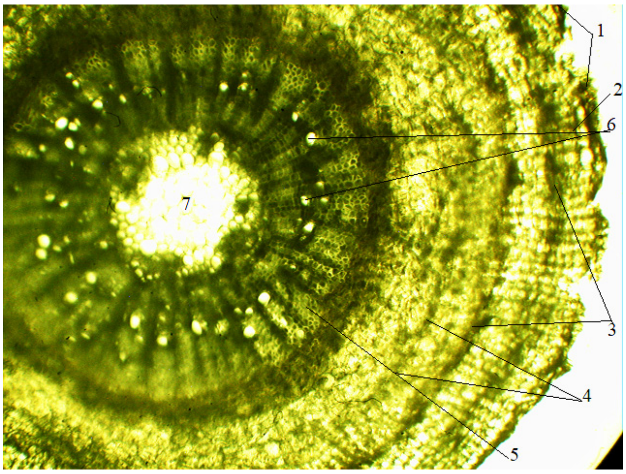

Figure 6 – Anatomical structure of *F. sogdiana* (Temirlik river) stem (x70). Note: 1-periderm, 2 – parenchyma of the primary cortex, 3-bast, 4-cambium, 5-sclerenchyma, 6-xylem vessels, 7-core

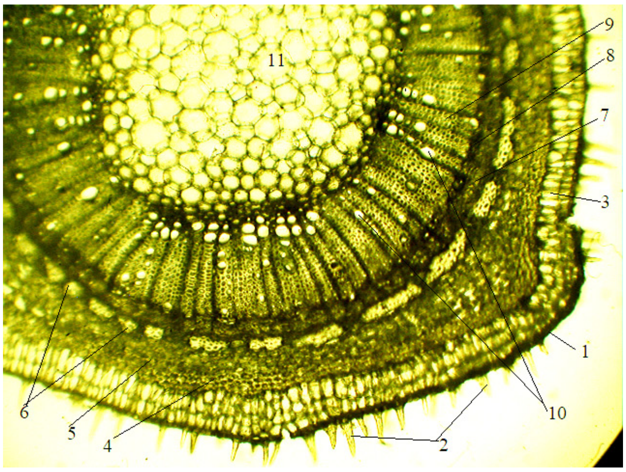

Figure 7 – Anatomical structure of *F. sogdiana* (Sharyn river) stem (x70). Note: 1-periderm, 2-simple hairs, 3-primary cortical parenchyma, 4-collenchyma, 5-parenchyma, 6-secondary phloem with groups of stony cells, 7-cambial zone, 8-secondary xylem, 9-sclerenchyma, 10-xylem vessels, 11-core parenchyma

Table 3 – Morphometric parameters of *F. sogdiana* stems

Area	Periderm thickness, mkm	Primary cortex thickness, mkm	Thickness of the sclerenchymal layer in the central cylinder, mkm	Diameter of the central cylinder, mkm
river Temirlik average value	0,061±1,9	2,225±0,7	0,341±0,2	41,096±12,2
river Sharyn average value	1,066±0,9	1,094±0,8	0,445±0,002	84,801±14,07

Anatomical and morphological structure of the leaf F. sogdiana. Regardless of the place of growth (site) leaf cross-sections of *F. sogdiana*, showed thickened outer walls of the cells of the upper and lower epidermis (1,2) with a thin layer of cuticle (Figure 8, 9). The cells of the epidermis are small, slightly oblong. The upper epidermis (1) is less thick than, the lower epidermis (2).

The stomata complex is located at the level of the epidermis, the air gaps under the stomata are visible. The mesophyll occupies the entire space between the upper (1) and lower epidermis (2). Mesophyll cells are fairly uniform in shape, with slightly elongated – columnar mesophyll (3) and, rounded – spongy mesophyll (4). The columnar mesophyll in plants from the Temirlik site consists of two rows of oblong cells, consisting of many chloroplasts (Figure 8). The transition to the spongy parenchyma is clear. Spongy parenchyma consists of 3-4 cell rows with large air spaces (8). Mesophyll consists of cells of columnar and spongy mesophyll. The thickness of the mesophyll is 0.610 µm. In plants from the area of p. The Sharyn mesophyll consists of three alternating rows of cells (columnar-spongy-columnar) (Figure 9). There are more photosynthetic elements in the columnar mesophyll. The thickness of the mesophyll is 0.514 µm.

The conducting bundles located directly in the mesophyll are surrounded by single cells of the sclerenchyma, whose cells are elongated along the bundle. In plants from both sites, the conducting bundles (5), are collaterally closed (Figure 8, 9). The bundles are represented by sieve-like tubes of the phloem (6) and vessels of the xylem (7).

The morphometric parameters of the leaves are presented in Table 4.

From the results shown in Table 4, it can be seen that the lower and upper epidermis, and leaf blades in *F. sogdiana* of the *Sharyn River* region are thicker than in plants of the Temirlik site. In contrary, the thickness of the mesophyll is smaller in plants of the *Sharyn River* compared to plants of the *Temirlik* site. According to our results, the area of conducting bundles was slightly larger in plants from the *Sharyn* site.

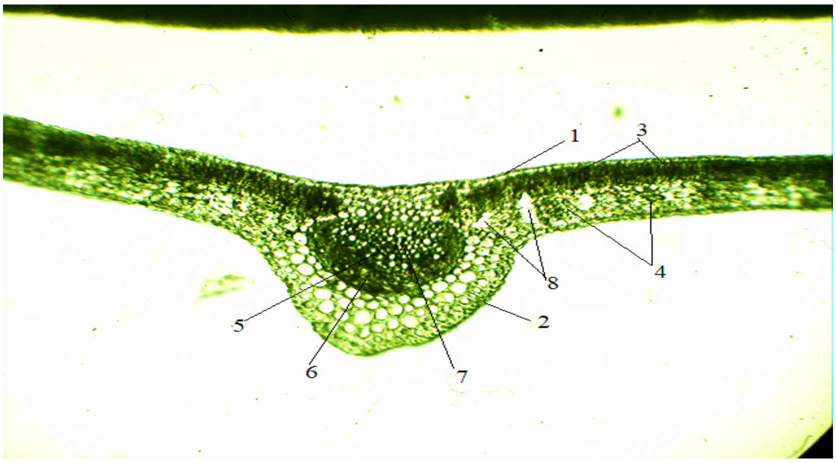

Figure 8 – Anatomical structure of *F. sogdiana* (Temirlik River) leaves (x100).
Note: 1-upper epidermis, 2-lower epidermis, 3-columnar mesophyll, 4-spongy mesophyll, 5-conducting bundle, 6-sieve tubes of phloem, 7-xylem vessels, 8- air spaces

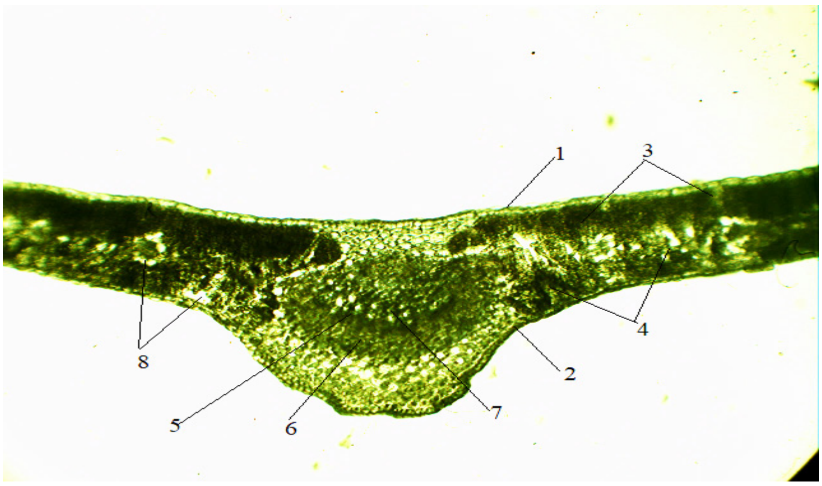

Figure 9 – Anatomical structure of *F. sogdiana* (Sharyn River) leaves (x100).
Note: 1-upper epidermis, 2-lower epidermis, 3-columnar mesophyll, 4-spongy mesophyll, 5-conducting bundle, 6-sieve tubes of phloem, 7-xylem vessels, 8-air spaces

Table 4 – Morphometric parameters of *F. sogdiana* leaves

Site	Thickness of the epidermis, mkm		Thickness of the leaf blade, mkm	Thickness of the mesophyll layer, mkm	Cross-sectional area of conducting bundles, x10^{3}mm^2
Average value	lower	upper			
Temirlik river	0,058±0,05	0,054±0,09	1,041±0,09	0,610±0,09	0,060±0,03
Sharyn river	0,067±0,06	0,071±0,03	1,277±0,6	0,514±0,7	0,080±0,04

Conclusion

Our results show differences in the root, stem, and leaf anatomy of young Sogdian ash trees growing at different sites (*Sharyn River* and *Temirlik River* area) in the Almaty region of Kazakhstan.

1. The primary cortex was twice as thick for ash trees from the *Sharyn River* area compared to ash trees from the *Temirlik River* area, probably due to the growing conditions, since the soil of the Sharyn River valley is meadow, stony and pebbly, compared to the Temirlik River, which is a deposit of red clay mixed with crushed stone and rock remnants.

2. The diameter of the central cylinder in the stem was twice as wide in *F. sogdiana* trees from the *Sharyn River* valley.

3. The thickness of the leaf blade as well as the lower and upper epidermis in leaves of *F. sogdiana* was larger at the *Sharyn River* area than at the *Temirlik River*, area.

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