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Comparative anatomical and morphological study of vegetative organs of *Gymnospermium altaicum* (Pall.) Spach from natural populations

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Abstract. Plants growing in arid environments developed structural adaptations to reduce water loss and dissipate excessive light energy. However, such adaptations as thick cuticle, sunken stomata, developed mechanical tissue, and trichomes, may have various functions and can also be found in plants growing in mesic environments. To better understand the role of xeromorphic adaptations, it is important to study plants of the same species from different habitats. The object of our study was *Gymnospermium altaicum* (Pall.), a small perennial plant having the status of a rare species for Kazakhstan. For anatomical and morphometric studies, plants were sampled from three populations with different moisture regimes. Populations 1 and 3 were growing in drier conditions, on south-east and north-east slopes, respectively, while population 2 was found on a north-facing slope receiving more rainfall. We demonstrate, for the first time, that anatomical features and morphometric parameters of *G. altaicum* are correlated to the humidity variations. The xeromorphic features of vegetative organs of plants from populations 1 and 3 were not observed in plants from population 2. The outcomes of this research will help us to better understand the adaptive potential of *G. altaicum* and develop effective conservation strategies for its wild populations.

1 Introduction

The study of the internal and external plant structure is timely, due to a growing demand for medicinal plants with valuable pharmacological properties. Multiple studies suggested that further research was needed in this area.

One of the little-studied plants is *Gymnospermium* represented in Kazakhstan by only two species, *G. alberti* (Regel) Takht. and *G. altaicum* (Pall.) Spach. Both species are confined mainly to the mountain broadleaf forests. *G. alberti* is found in the mountain ranges of Southern Kazakhstan, and *G. altaicum* in the mountain ranges in the southeast and east of the republic. The latter species is a rare plant in Kazakhstan [1-4].

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Anatomical characteristics of plants are conserved and stable and can therefore be used in taxonomic characterization as well as for assessing the adaptation of plants to different environmental conditions. This is especially relevant to rare and endangered species, which are of great research interest for the researchers. *Cladium mariscus* (L.) Pohl. – a rare protected species which adaptive potential and resistance to unfavorable habitat conditions were assessed by studying the microscopic structure of the root, rhizome, and leaf [5]. The anatomical features of vegetative organs and the phytochemical composition of rare species of Kazakhstan, *Fraxinus sogdiana* Bunge (Oleaceae), were studied by some authors in different soil conditions in the river valley of Temerlik [6]. *Aethionema lepidioides* Hub. – an endemic endangered plant in Turkey was investigated to determine the morphological parameters of its vegetative and generative organs [7]. To the precise taxonomic determination of the medicinal plant *Prunus africana* the structural features of its vegetative organs and seeds were studied [8]. Also, to understand the adaptive significance of morpho-anatomical reactions to the natural habitat of the medicinal plant *Eugenia luschnathiana* (O. Berg) (Myrtaceae), the leaf anatomy was described in wet and dry seasons, as well as in sun and shade [9].

From the above, it can be inferred that rare, endangered species of medicinal plants are of great research interest in terms of their anatomy.

2 Materials and Methods

The object of our study was *Gymnospermium altaicum,* an ephemeroid geophyte of the family Berberidaceae Juss.. The plant material was collected from three permanent sample plots in Trans-Ili Alatau [10]:

1) Population 1 – Kotyr-Bulak gorge, hawthorn forest, slope bottom (altitude 952 m);
2) Population 2 – Kuznetsovo tract (Tau Turgen Gorge), apple forest with mixed herbs (altitude 1550 m);
3) Population 3 – Soldatsai Gorge, apple forest with grasses and mixed herbs (altitude 1540 m).

To study anatomical features of the *G. altaicum* roots, stems and leaves, we used the accepted methods [11-13].

The following equipment was used: a microtome with a freezing device OL-ZSO (Inmedprom, Russia); an MOV-1-15 eyepiece micrometer (with a lens of x10 and magnification of x40, 10, and 7); and an MC 300 microscope (Micros, Austria) with a CAM V400 / 1.3M video camera (jProbe, Japan).

3 Results and Discussion

G. altaicum leaf blades had an elongated ovoid shape. On the upper side of the leaf, epidermal cells were large, thin-walled, cuticle-free, and had single stomata. Numerous slightly immersed stomata were on the abaxial surface of the lamina. The oblong-round cells of the epidermis were stretched along the leaf proximodistal axis. There were one (populations 1 and 3) or two (population 2) rows of the mesophyll cells under the adaxial epidermis, which were slightly oval-shaped, thin-walled parenchyma cells with sparse intercellular spaces between them. Bordering the abaxial epidermis, the cells of mesophyll had less size and more chloroplasts than the cells in the adaxial part of the leaf. There was the spongy mesophyll on the abaxial part of the leaf, and the air-bearing cells of the main leaf tissue were under the vascular bundles. The shape and size of the air-bearing cells of the main tissue were different in plants collected from different populations. Thus, in plants from population 1, air-bearing cells were large and rounded, their average number was 15-

20. In plants from population 2, the number of air-bearing cells was much smaller, 2-3. In plants from population 3, air-bearing cells were rounded. They were adjacent to the basal part of the vascular bundle, and their average number was 5-6.

Closed collateral conductive bundles were located inside the mesophyll**.** The conductive bundles that directly passed through the central part of the leaf were the biggest of them all and were enclosed by solitary cells of sclerenchyma, while smaller bundles were immersed in the mesophyll. This was clearly seen in population 3. Inside the mesophyll, cells containing inclusions were noted (Figure 1). Table 1 presents morphometric parameters of *G. altaicum* leaf blades from the three populations studied.

Table 1. Morphometric parameters of leaves of *G. altaicum* (Pall.) Spach (mean values).

Population	Epidermis thickness, µm		Mesophyll thickness, µm	Columnar mesophyll thickness, µm	Spongy mesophyll thickness, µm	Area of the cross-sections of conductive beams, x10^{-3}mm^2
	upper	lower				
1	0.35±0.04	0.36±0.06	12±0.961	4.5±0.2	4.8±0.1	30.38±0.08
2	0.46±0.03	0.30±0.07	18.2±0.3	8.1±0.4	9.1±0.4	30.03±0.02
3	0.44±0.08	0.4±0.1	11.6±0.4	5.2±0.4	4.3±0.3	30.18±0.03

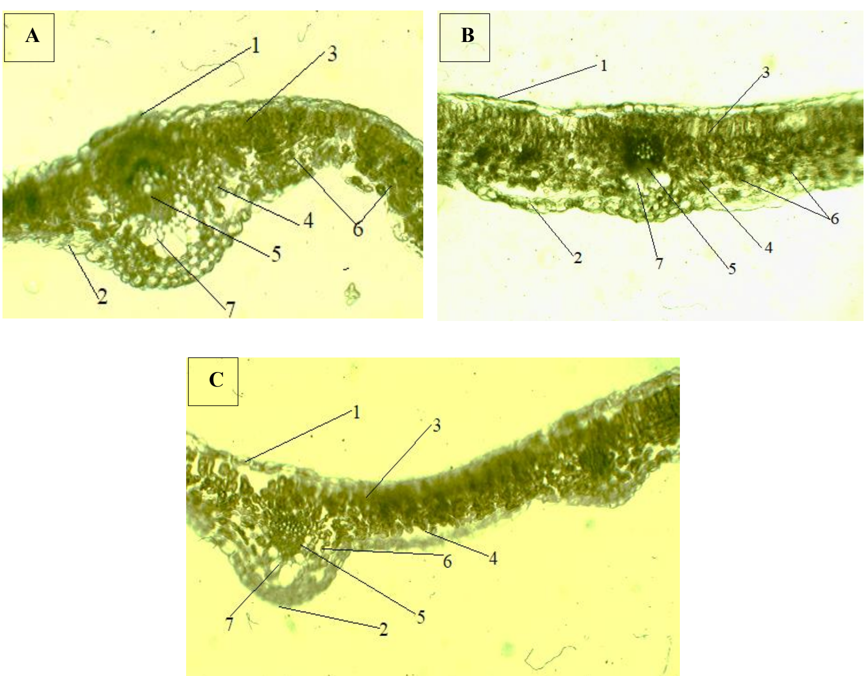


Fig. 1. Anatomical structure of the leaf blade of *G. altaicum* (x100). A- population 1 (Kotyr-Bulak Gorge); B- population 2 (Tau Turgen Gorge); C- population 3 (Soldatsai Gorge). 1 – upper epidermis, 2 – lower epidermis, 3 – palisade mesophyll, 4 - spongy mesophyll, 5 – conductive bundle, 6 – inclusions, 7 – air-bearing cells of the main leaf tissue

On the cross-section, the *G. altaicum* stem was rounded, slightly ribbed, with a weak pubescence associated with the habitat ecological conditions. The external wall of the epidermal cells was covered by a thin layer of the cuticle, on which simple and glandular hairs were visible. The largest number of epidermal formations was found in plants from population 1. The primary cortex was represented by the cells of the primary cortex and endoderm. At high magnification, it could be seen that the chlorenchyma made the main part of the primary cortex. In population 3, the smallest number of chlorophyll-bearing cells was observed in the primary cortex. The cells of the primary cortex were numerous, loosely arranged. It would be worth mentioning here in the populations 1 and 2, the element of primary cortex was more pronounced and represented by 3-4 layers of cells, while in population 3, the chlorenchyma cells were located more loosely and contained the largest number of inclusions. The inner row of the larger cortical parenchyma cells is called the endoderm. The sclerenchyma cells occurred singly or in groups. From the endoderm inwards, the entire space was occupied by the main parenchyma with scattered vascular bundles. The vascular bundles were randomly arranged. Larger bundles alternated with smaller ones. There were more bundles on the periphery, and fewer, in the center of the stem. The sclerenchyma cells were most developed in plants of populations 1 and 3, and least developed in population 2. The conductive bundles were closed collateral (Figure 2). Table 2 presents morphometric parameters of the stems of *G. altaicum* from the three populations studied.

Table 2. Morphometric parameters of *G. altaicum* stems (mean values).

Population	Primary cortex thickness, µm	Conductive bundle diameter, µm	Central cylinder diameter, µm
1	7.1±0.1	31±2	78.621
2	7.2±0.3	37±5	80.697
3	9.0±0.6	30±4	78.181

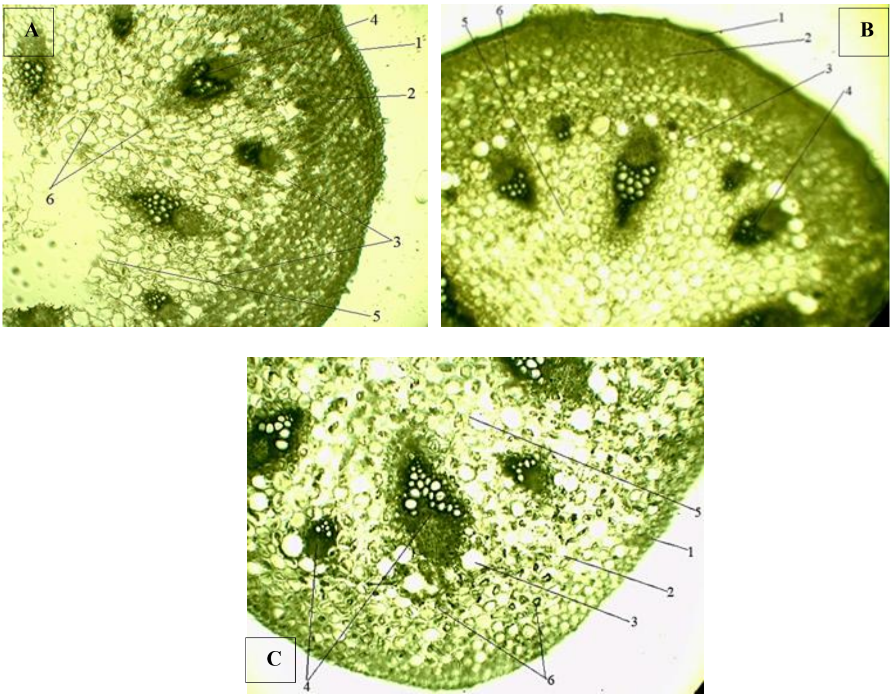
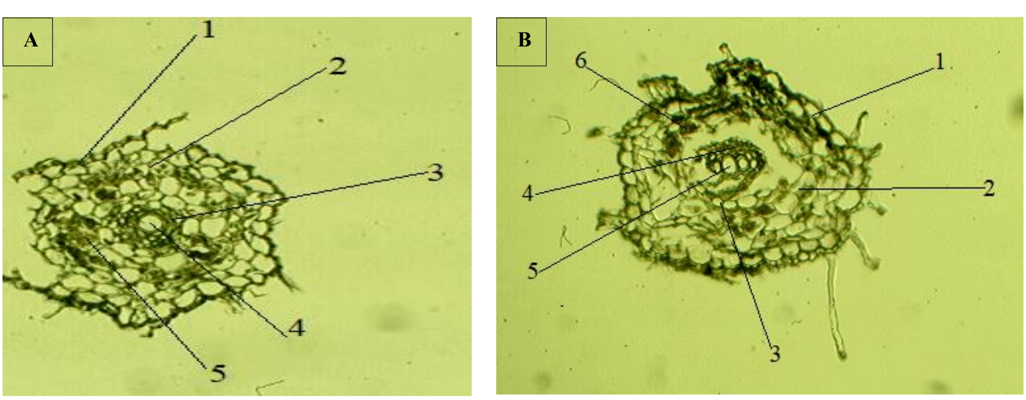


Fig. 2. Anatomical structure of the stem of G. altaicum (x100). A- population 1 (Kotyr-Bulak Gorge); B- population 2 (Tau Turgen Gorge); C- opulation 3 (Soldatsai Gorge). 1 – epidermis, 2 – primary cortex, 3 - endoderm, 4 – conductive bundles, 5 – core cells, 6 – inclusions

The anatomical structure of the root is presented in Figure 3. On the cross-section, at x140 magnification a relatively small central cylinder is visible. The primary cortex is covered with a single layer of the ectoderm, on the surface of which single residues of the rhizoderm cells are visible. The ectoderm consists of tightly closed oval cells somewhat elongated in the radial direction. The primary cortex is loose. It consists of large parenchyma cells with slightly thickened walls and numerous intercellular spaces of different shapes.

The inner part of the root was occupied by a central cylinder. The xylem was represented by one (population 1) or two (populations 2 and 3) xylem vessels. The primary phloem consisting of sieve and satellite cells was located around the xylem vessels. The number of vessels in the three populations of *G. altaicum* differed according to the differences in the habitat moisture regime.



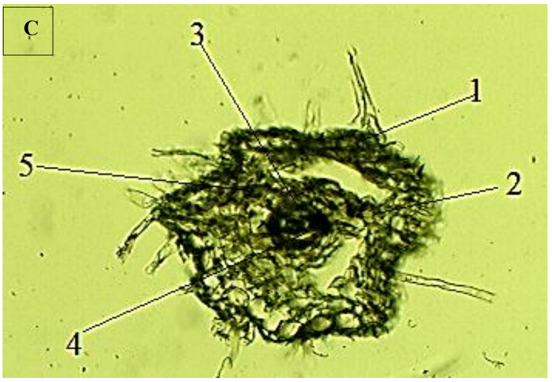


Fig. 3. Anatomical structure of the root of *G. altaicum* (x140). A- population 1 (Kotyr-Bulak Gorge); B- population 2 (Tau Turgen Gorge); C- opulation 3 (Soldatsai Gorge). 1 – exoderm, 2 – primary cortex, 3 - phloem, 4 - xylem vessel, 5 - inclusions

Table 3 presents morphometric parameters of the roots of *G. altaicum* from the three populations studied.

Table 3. Morphometric parameters of *G. altaicum* roots (mean values).

Population	Primary cortex thickness, µm	Central cylinder diameter, µm	Xylem vessel diameter, µm
1	3.8±0.6	6±2	0.9±0.5
2	4.2±0.3	7±1	0.7±0.1
3	3.8±0.5	6±2	0.8±0.6

4 Conclusion

The results of our study confirm that the plants of *G. altaicum* collected from the three populations studied differed in their anatomical characteristics. Specifically, the plants growing in dryer conditions (populations 1 and 3) had xeromorphic features, while the plants from population 2 were mesomorphic. The following structural adaptations to reduce water loss in plants from dryer habitats (populations 1 and 3) were observed: in the roots, the presence of sclerenchyma cells and inclusions, and thickened exoderm cells; in the leaves, more dense cuticle, sunken stomata, numerous fine cells in the spongy mesophyll, and densely packaged cells without intercellular spaces in the palisade mesophyll. In the leaf lamina of mesomorphic plants (population 2), we watched loose spongy parenchyma with a pattern of intercellular spaces, as well as more shallow and less frequent vascular bundles. The stems and their vascular bundles were larger in term of size, and the number of chlorophyll-bearing cells in the stem were more than those in other populations. In the roots, we observed a thicker primary bark and increased diameter of the central cylinder.

Thus, a comparison of *G. altaicum* plants from drier habitats with those from mesic environment suggests that the observed structural adaptations to xeric environment are xeromorphic features formed in response to limited water availability and excessive insolation.

Authors' contribution

D. Almabek, K. Abidkulova, B. Turalin executed the study, N. Kurbatova, N. Mukhitdinov interpreted the study and prepared the manuscript.

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