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Study of anatomical structure of *Dactylorhiza fuchsii* (Orchidaceae) growing in the Karkaraly Mountains (Central Kazakhstan)

The study of plant structure peculiarities plays an important role in understanding the ecology and habitat conditions of species, as well as for the identification of characteristic features in confirming the species identity of plant raw materials. This article presented the results of microscopic structure of above-ground and underground organs of *Dactylorhiza fuchsii* growing in the Karkaraly Mountains (Central Kazakhstan). The results showed that the leaf preparation is characterized by the presence of polygonal epidermal cells, stomata of tetracytic type, predominantly localized on the lower side of the leaf. Mesophyll is of homogeneous type, bundles are collateral, cranium-lined, and usually oval in shape. The stem on transverse section is characterized by a well-defined epidermis and cortex, numerous conductive bundles, collateral type, and several lacunae are presented. The transverse section of the root is characterized by the presence of starch grains and fungal peletons, indicating the symbiosis of this species with fungi under natural conditions. In general, the features of the anatomical structure confirm the mesophytic character of *Dactylorhiza fuchsii* habitat, in particular, the absence of velamen in the root and adaptations in the leaves for water absorption. The results obtained contribute to the assessment of ecological and biological peculiarities of the species and allow us to use the obtained characters for taxon identification at the microscopic level.

Keywords: *Dactylorhiza fuchsii*, anatomical structure, Karkaraly Mountains, leaf, stem, root, mesophytic signs.

Introduction

Orchids are among the most vulnerable plants protected internationally. All species of the Orchidaceae family are listed in the Annex of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). *Dactylorhiza fuchsii* (Druce) Soo is protected at the national level in Belgium and Luxembourg, and at the regional level in France [1]. In the Russian Federation, this species is included in the Red Data Books of most regions. In Kazakhstan, *Dactylorhiza fuchsii* is included in the Red Data Book [2] as the only representative of the genus *Dactylorhiza*. In the Central and Northern Kazakhstan it is found in such protected areas as Kokshetau, Karkaraly, and Burabai national parks [3, 4].

Like many representatives of the family Orchidaceae, the abundance of *Dactylorhiza fuchsii* in nature is decreasing due to anthropogenic impacts, including habitat destruction, climate change and plant harvesting. In this regard, study of the anatomical features of this species become relevant, as they allow us to understand the mechanisms of its adaptation to different environmental conditions. It is worth noting that the roots and rhizomes of *Dactylorhiza fuchsii* contain biologically valuable substances and can be active against infectious agents [5].

International studies of morphology and micromorphology of orchids are actively conducted in Europe [6]. However, the anatomy of *Dactylorhiza fuchsii* remains insufficiently studied [7, 8], and in Kazakhstan, this kind of research is generally not conducted.

Anatomical studies cover such aspects as root system structure, stem morphology, leaf composition and features of floral structures. Root tubers play an important role in nutrient accumulation, the stem provides support and transport of water and minerals, and leaves are adapted for optimal photosynthesis. Specialized floral structures indicate complex relationships with pollinators [9–11].

The aim of this study is to investigate the anatomical features of *Dactylorhiza fuchsii* to understand the mechanisms of its adaptation to the environment. The results of the study can be used to develop strategies for the conservation of the species, effective management of its populations and restoration of natural habi-

tats. In addition, the study of the anatomy of *Dactylorhiza fuchsii* contributes to the expansion of scientific knowledge in the field of botany and plant physiology, and can also be used to clarify the diagnostic features of medicinal plant raw materials.

Experimental

The object of the study were aboveground and underground organs of *Dactylorhiza fuchsii* (root tubers, leaves, shoots) collected in the 2nd decade of July 2024 in the Karkaraly Mountains (Karkaraly district, Karaganda region) in the flowering phase (Fig. 1). Sampling coordinates for the study: N49°19'54.174604“, E75°33'11.665018”, 893 m above sea level.

Figure 1. Flowering samples of *Dactylorhiza fuchsii* in Karkaraly Mountains (2024)

Fresh plants were fixed in a mixture of ethyl alcohol 96 % — distilled water — aqueous glycerol 40 % (1:1:1 ratio, Strauss-Fleming reagent). Transverse sections and surface preparations were made manually using a blade [12–14]. The middle part of the shoot was used for the stem, the central vein for the leaf, and the central part for the petiole. The obtained micro specimens were viewed through a binocular microscope NINGBOSUNNY Instruments CoEX30 (China) with a digital camera Altami 8.5 Mpix (Russian Federation). AltamiStudio software was used for photo processing. When describing the anatomical structure we used the principles outlined in the works of L.I. Lotova [15] and P.J. Rudall [16].

Results and Discussion

Dactylorrhiza fuchsii is a perennial herbaceous plant, 15–25 cm tall; tubers are rounded or turnip-shaped, palmate. Shoots are solitary, dense, 5–7 mm in diameter. Leaves are few, 2 to 5 in number, slightly deviated from the stem, almost flat or folded along the central vein, oblong-lanceolated in the lower part and awl-shaped-linear and slightly sinuous in the upper part of the stem; color is green with rounded purple spots of different sizes. Flowers are gathered in spike-like inflorescences, up to 8-9 cm long and 3-4 cm in diameter. The color of flowers varies widely, from white to purplish-red.

Microscopic examination of the leaf (Fig. 2) showed that differences in the structure of the upper and lower sides of the plate were observed.

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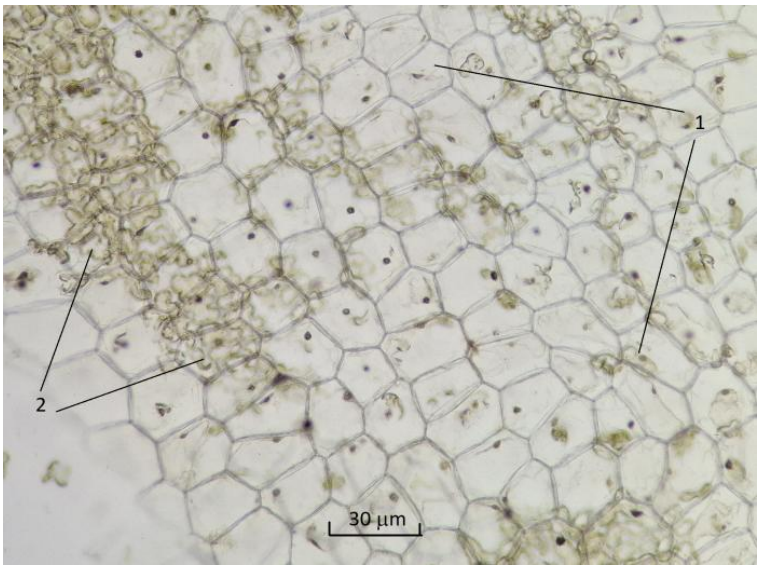


A

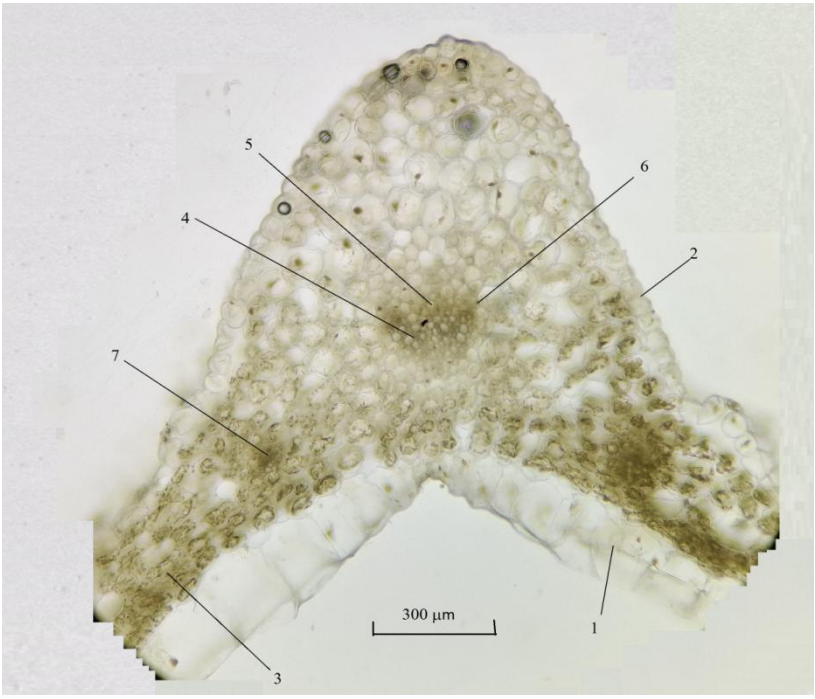
B

1 — basic cells of epidermis, 2 — leaf mesophyll cells, 3 — stomata; A — upper epidermis, B — lower epidermis

Figure 2. Preparation of *Dactylorhiza fuchsii* leaf from surface

The epidermis of the upper side consists of thin-walled polygonal cells, rounded or slightly oval in shape, through which the polygonal cells of the mesophyll are well translucent. The size of the cells ranges from 20 to 35 µm. Cells of the lower epidermis are smaller in size, 20–30 µm, with weakly curved walls, slightly elongated along the central vein. Nuclei are well expressed in the epidermal cells of both sides of the leaf. Stomata are identified only on the lower side (hypostomal type). Stomata are rounded, 15–20 µm in diameter, tetracytic type. Such features of the structure were confirmed in the works of L.V. Averyanov [17].

The internal structure of the leaf (Fig. 3, 4) is characterized by a homogeneous type of mesophyll structure, which is reflected in the absence of its differentiation into palisade and spongy tissues; leaf thickness in the lateral part is 250–305 µm, in the region of the central vein — up to 400 µm.



1 — upper epidermis, 2 — lower epidermis, 3 — mesophyll, 4 — xylem,
5 — phloem, 6 — cranial lining, 7 — lateral vascular bundle

Figure 3. Leaf preparation of *Dactylorhiza fuchsii*, transverse section, fragment in the area of the central vein

1 — upper epidermis, 2 — lower epidermis, 3 — mesophyll, 4 — lateral vascular bundle

Figure 4. Leaf preparation of *Dactylorhiza fuchsii*, transverse section,
fragment in the area of the lateral part of the leaf lamina

The total number of cell layers ranges from 7 to 9. This structure is typical for many species of Orchidaceae [18, 19]. The epidermis is single-layered, consisting of isodiametric and almost square cells with a small amount of intercellular space; the cuticle is absent on both sides. The cells of the upper side are larger in size than those of the upper side; the shape on the transverse section is similar. Leaves have collateral vascular bundles; phloem is oriented toward the abaxial side, xylem toward the adaxial side. The xylem is poorly differentiated. A cranial lining is formed around the bundles. Sclerenchyma fibers are almost not observed.

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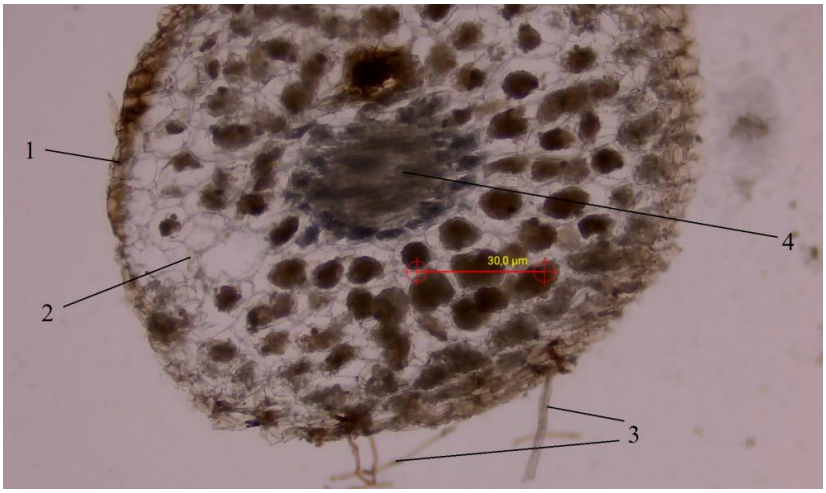
On anatomical sections of the stem, a rounded or ovoid shape with remnants of adjacent leaves can be observed (Fig. 5). The perimeter of the stem is covered with one-layer epidermis with a thin cuticular layer; the shape of cells is oval.

1 — leaf area adjacent to the stem, 2 — epidermis, 3 — cortex,
4 — medullary parenchyma, 5 — xylem, 6 — lacuna

Figure 5. Cross section of leaf of *Dactylorhiza fuchsii*

The epidermis is followed by the cortex, consisting of parenchymatous cells, in 10–15 layers. Part of the cells is filled with starch granules. The central part of the stem is filled with medullary cells. Vascular bundles are collateral, reminiscent of dicotyledonous plants and arranged at regular intervals. Elements of xylem are well visible; elements of phloem are almost invisible due to thin cell walls. There are several lacunae in the stem due to the core being broken into parts. These features are also characteristic of other Orchid species *like Ophyslutea* and *Orchismascula* [20, 21].

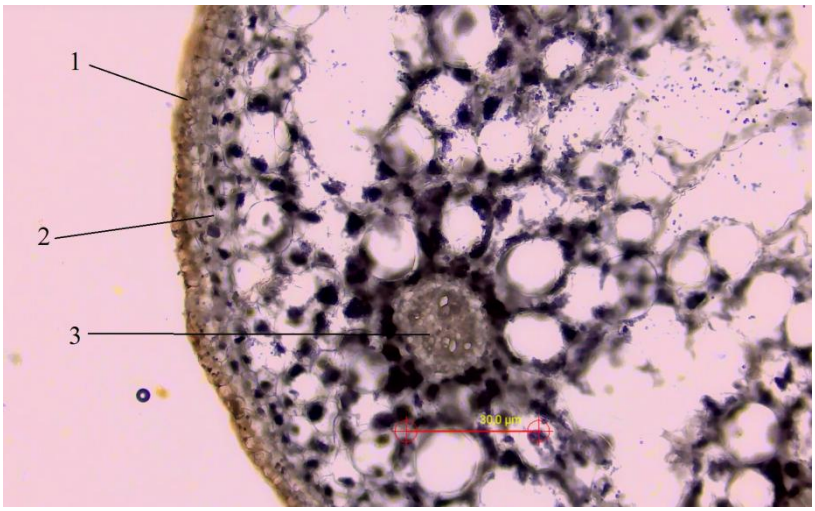
Examination of root anatomy showed that the outer layer consists of 1-2 layers of rhizoderm, the cells of which do not have a stable shape. The cells can be elongated in longitudinal or transverse direction, sometimes rounded in shape. The cells of the cortex consists of 5–8 layers, usually rounded, their size decreases towards the central part. Remains of fungal peletons can be noted on the outside [22]. The central part of the stem is occupied by a vascular bundle with sclerenchyma lining.



1 — rhizoderm, 2 — cortex, 3 — fungal peletons, 4 — vascular bubdle

Figure 6. Cross section of *Dactylorhiza fuchsia* root

Transverse section of root tuber anatomy showed the presence of starch grains in cells when stained with Lugol's solution (Fig. 7). The endoderm is 1-layered, completely surrounding the meristem periphery. Vascular bundles are several in number; arranged in a circle, as in dicotyledonous plants. The bundles consist of xylem, sieve tubes, phloem parenchyma, cambium is absent; there are some areas of pericycle.



1 — rhizoderm, 2 — endoderm, 3 — vascular bundle

Figure 7. Cross section of root tuber of *Dactylorhiza fuchsii*. Fragment

Conclusion

Thus, according to the peculiarities of structure, *Dactylorhiza fuchsii* belongs to mesophytic tuberous-rooted species forming symbiosis with fungi. In general, the structure of above-ground and underground organs shows the presence of adaptations for water absorption and retention as a system of bark parenchyma (cortex). The absence of the velamen which is formed in many genera of Orchidaceae is noted. Leaves of the plant do not have adaptations for water retention, which indicates growth in conditions of sufficient moisture without the test of constant hydric stress.

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Қарқаралы (Орталық Қазақстан) тауларында өсетін *Dactylorhiza fuchsii* (Orchidaceae) анатомиялық құрылымын зерттеу

Өсімдіктер құрылымының ерекшеліктерін зерттеу түрдің экологиясы мен тіршілік ету ортасын түсінуде, сондай-ақ өсімдік шикізатының түрлерін растау кезінде тән белгілерді анықтауда маңызды рөл атқарады. Мақалада Қарқаралы (Орталық Қазақстан) тауларында өсетін *Dactylorhiza fuchsii* жерүсті және жерасты органдарының микроскопиялық құрылысының нәтижелері келтірілген. Нәтижелер жапырақ препараты негізінен жапырақтың төменгі жағында локализацияланған эпидермистің көпбұрышты жасушаларының, тетрацит типті саңылауларының болуымен сипатталатынын көрсетті. Мезофилл біртекті типтес, шоғырлары бүйірлі, сақина тәріздес, сопақша пішінді. Көлденең кесілген сабақ айқын анықталған эпидермис және қыртыспен сипатталады; коллатеральды типтегі көптеген өткізгіш шоқтарымен, сонымен қатар бірнеше қуыстары бар. Көлденең кесілген тамыр крахмал дәндері мен саңырауқұлақ пелетондарының болуымен сипатталады, бұл табиғи жағдайда саңырауқұлақтармен осы түрдің симбиозын көрсетеді. Тұтастай алғанда, анатомиялық құрылымның ерекшеліктері *Dactylorhiza fuchsii* тіршілік етуінің мезофиттік сипатын растайды, атап айтқанда, бұл тамырда веламеннің болмауы және суды сіңіру үшін жапырақтардың бейімделуі. Алынған нәтижелер түрдің экологиялық-билогиялық ерекшеліктерін бағалауға ықпал етеді және алынған белгілерді таксонды микроскопиялық деңгейде анықтау үшін пайдалануға мүмкіндік береді.

Кілт сөздер: *Dactylorhiza fuchsii*, анатомиялық құрылымы, Қарқаралы таулары, жапырақ, сабақ, тамыр, мезофиттік белгілері.

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Изучение анатомической структуры *Dactylorhiza fuchsii* (Orchidaceae) произрастающей в горах Каркаралы (Центральный Казахстан)

Изучение особенностей строения растений играет важную роль для понимания экологии и условий обитания вида, а также для выделения характерных признаков при подтверждении видовой принадлежности растительного сырья. В настоящей статье приведены результаты микроскопического строения надземных и подземных органов *Dactylorhiza fuchsii*, произрастающей в горах Каркаралы (Центральный Казахстан). Результаты показали, что препарат листа характеризуется присутствием многоугольных клеток эпидермиса, устьица тетрацитного типа, преимущественно локализованы с нижней стороны листа. Мезофилл гомогенного типа, пучки — коллатеральные, с кранц-обкладкой, обычно овальной формы. Стебель на поперечном срезе характеризуется четко выраженным эпидермисом и кортексом, присутствуют многочисленные проводящие пучки, коллатерального типа, а также несколько лакун. Поперечный срез корня характеризуется присутствием крахмальных зерен и грибных пелетонов, что свидетельствует о симбиозе данного вида с грибами в природных условиях. В целом, особенности анатомического строения подтверждают мезофитный характер обитания *Dactylorhiza fuchsii*, в частности, это отсутствие веламена у корня и приспособлений у листьев для поглощения воды. Полученные результаты вносят вклад в оценку эколого-билогических особенностей вида и позволяют использовать полученные признаки для идентификации таксона на микроскопическом уровне.

Ключевые слова: *Dactylorhiza fuchsii*, анатомическое строение, горы Каркаралы, лист, стебель, корень, мезофитные признаки.

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