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RESEARCH ARTICLE

Anatomical and morphological features of leaves of *Rhodiola rosea* L. in natural conditions of the mountains in Southern Siberia (Altai Mountains)

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Abstract

The study of natural coenopopulations of the rare medicinal species *Rhodiola rosea* is of highest relevance for conservation of this valuable plant species. Analysis of the adaptive response of the species growing under different environmental conditions is basically used to elaborate recommendations for its protection and rational use. The aim of this work was to study the anatomical and morphological features of the leaf blades of *Rh. rosea* growing under different environmental and coenotic conditions of the Altai Mountains. We studied the anatomical features of leaves of *Rh. rosea* from 7 coenopopulations (in one coenopopulation, both female and male plants were studied) and assessed the adaptive response of plants to different growing conditions by conventional methods. The values of indicators from different coenopopulations were compared by one-way analysis of variance (ANOVA), and the correlation coefficient was calculated to estimate the correlation between different indicators. The study revealed a reserve of adaptive plasticity for *Rh. rosea*, which allows the species to grow under different light and water conditions. For specimens from one geographic location studied, statistically significant differences in the indicator values were fewer compared with specimens from different areas. Heliophytic and xerophytic adaptations of the studied plants varied depending on coenopopulation. The analysis of male and female *Rh. rosea* species from the Kurai ridge (CP 3) revealed that the most photosynthetically active layer and vascular tissue are better developed in leaves of females compared to males. It was found that *Rh. rosea* growing in the mountains bear shorter and thicker leaves; the thickness of integumentary tissue with cuticle increases at higher altitudes, the number of stomata and cells on integumentary tissue per epidermis unit area decreases, and the thickness of photoassimilating tissue grows. Changes in anatomical and morphological characteristics are adaptive responses to increased insolation at higher altitudes and, probably, to decreased partial pressure of carbon dioxide.

Keywords
Adaptive plasticity, coenopopulations, environment, Gorny Altai, leaf anatomy, *Rhodiola rosea*

Introduction

Rare species are particularly vulnerable and sensitive to the natural environment. These plants include species that possess valuable economic properties (medicinal, ornamental, food). Comprehensive studies of their natural coenopopulations are critical to preservation of valuable flora species. Knowledge of the response of the rare species to adverse environmental effects can help elaborate measures for its protection and rational use (Semenova 2007; Magomedmirzaev et al. 2013; Zlobin et al. 2013). The same plant species in different conditions, represented by specific populations, can implement different life strategies (Blinova 2014; Poliakova 2022). Adaptive parameters of species can be used to develop technologies for bioindication, biotesting and environmental forecasting (Popova 2011; Legoshchina et al. 2013; Chadaeva and Shhagapsoev 2016; Kopanina et al. 2017). Conducting such studies is most effective when using an approach that covers several levels of organization of living systems: cellular, organ, organismal, population and coenotic (Oleinikova 2016; Ishmuratova 2020).

Representatives of the genus *Rhodiola* are valuable medicinal plants (Krasnov et al. 1979; Satsyperova et al. 1991; Belenovskaya and Medvedeva 2009). In natural coenopopulations, *Rhodiola* species are harvested as the medicinal raw material. Demand for these plants triggers alarm over uncontrolled harvesting, which may result in intensive reduction in the species numbers and disruption of coenopopulations. Many species of the genus *Rhodiola* are therefore included in the regional red books, and *Rh. rosea* is also protected at the federal level (Kozlov 2023).

High practical significance and conservation status necessitate the study of *Rh. rosea* in natural conditions (Nukhimovsky 1997; Frolov and Poletaeva 1998; Kim 1999; Nekratova and Nekratov 2005; Golovko et al. 2007; Prokopyev et al. 2021; Prokopyev et al. 2023; Dorogina et al. 2023). The analysis of biological and anatomical and morphological features of the species is one of the most relevant aspects to assess its adaptive potential in different environmental conditions.

The anatomical and morphological features of the vegetative organs of *Rh. rosea* have been studied in some regions of its natural growth and cultivation. E.F. Kim (1999) analyzed the anatomy of *Rh. rosea* leaves within the study of plant physiology under different water regimes. M.M. Ishmuratova (2006) reported changes in anatomical characteristics of the closely related species *Rh. iremelica* Boriss. with regard to the natural and climatic conditions of the habitat of each specific coenopopulation at higher altitudes and shift in the range from south to north. T.K. Golovko et al. (2007) showed that adaptive changes in the anatomical structure of leaves are stable in *Rh. rosea* species cultivated under similar conditions, but of different origin (Arctic and Ural plants). D.-I. Maftei and D.-E. Maftei (Maftei and Maftei 2015; 2018; 2019) studied the possibility of growing *Rh. rosea* in vitro, and concluded that the anatomical structure of the in vitro provided plants of *Rh. rosea*, post acclimatization and embedding in their native habitat, is similar to the one of the individuals from the spontaneous flora, their physiological activity being regular. The anatomical structure of rhizomes and stems, as well as the morphology of leaves of plants growing in the natural conditions of the Ceahlău mountains, does not have significant differences with plants grown in vitro and subsequently acclimatized in their native environment, or the differences are only quantitative and not qualitative. At the same time, the mechanical tissues of leaves and stems in plants grown in vitro are less developed or even absent. The conductive tissues are less developed in the in vitro plants, compared to those plants harvested from the native habitat. M.Zh. Zhumagul et al. (Zhumagul et al. 2020) studied the anatomical structure of leaves, stems and rhizomes, as well as the localization of biologically active substances in various plant structures. N.Y. Shmakova and O.V. Ermolaeva (2021) provided data on the mesostructure and functional activity of the photosynthetic apparatus of leaves of *Rh. rosea* grown in the Polar-Alpine Botanical Garden, Murmansk region. They found that the species is adapted to cold climatic conditions, and revealed the signs of plant adaptation to water deficit and increased insolation.

The aim of this work was to study the anatomical and morphological features of the leaf blades of *Rh. rosea* growing under different environmental and coenotic conditions of the Altai Mountains.

Materials and methods

The study object was a rare species with valuable medicinal properties – *Rhodiola rosea* or golden root (*Rhodiola rosea* L.) (Fig. 1).

Rhodiola rosea is a herbaceous short-rhizome polycarpic with an elongated erect shoot and succulent leaves, a hemicryptophyte (Bezdelev and Bezdeleva 2006; Goncharova 2006).

This arctic-alpine species with a disjunctive Eurasian range grows in the mountains of Western Europe, the Balkan Peninsula, Scandinavia, Asia Minor and Central Asia, Mongolia, China, Japan, and North America (Peshkova 1994; Kozhevnikov 2007). A significant part of the range covers the mountains of Southern Siberia, where the species is common in the subalpine and alpine mountain zones; along river banks, it descends into the forest belt (Polozhiy et al. 1985).

The study material was collected in the territory of Southern Siberia within the Altai Mountains (Chemalsky and Kosh-Agachsky regions, the Altai Republic). Desk studies were conducted in Siberian Botanical Garden, TSU (Tomsk).

Previously, we studied 9 coenopopulations of *Rh. rosea* from the Altai Mountains. The phytocoenotic confinement, demographic composition of coenopopulations, and morphological and reproductive features of the species were established (Prokopyev et al. 2021; Prokopyev et al. 2023).

Figure 1. *Rhodiola rosea* (male flower).

The anatomical features of leaves of the female *Rh. rosea* from 7 coenopopulations were studied (Table 1). For one of the coenopopulations (CP 3), the anatomical features of leaves of both male and female plants were compared and analyzed.

To compare the data with those obtained earlier, the numbering of coenopopulations was intentionally preserved in accordance with that provided in (Prokopyev et al. 2021; Prokopyev et al. 2023).

The anatomical structure of *Rh. rosea* leaves was studied using conventional methods (Vehov et al. 1980) based on studies by K. Ezau (Ezau 1980a, 1980b), C.S. Karataeva, A.S. Dariev and A.A. Pautov (Pautov 2003; Pautov 2012), M.A. Baranova (Baranova 1985), and S.F. Zakharevich (Zakharevich 1954); the stomatal index was calculated using A. Kästner's formula (Kästner 1972). Environmental adaptation of plants to different growing conditions was assessed based on studies by N.K. Boardman (Boardman 1977), T.K. Goryshina (Goryshina 1979), T.J. Givnish (Givnish 1988) and L.A. Ivanova (Ivanova 2014). In each of the studied coenopopulations, 1–2 leaves were taken from the middle part of the most developed shoot in 25 generative female plants and fixed in 70% ethanol. In CP 3, leaves of male plants were also collected for comparative analysis. Temporary preparations of fresh leaves were prepared by cutting made on a freezing microtome MZ-2 (Tochmedpribor, Ukraine). Five cross sections were made in the middle part of each leaf; the one of the highest quality was analyzed for morphometry. In total, not less than 30 sections were analyzed for each coenopopulation. The section thickness varied from 75 to 100 μm. The epidermis was cut with a razor from the middle third of the blade between the leaf edge and the central vein. Leaf microscope slides were taken and microscopic measurements were made using an Axio Lab. A1 microscope (Carl Zeiss, Germany) supplied with AxioCam ERc 5s digital camera. Anatomical and morphological characteristics were determined using Axio Vision 4.8 software. Anatomical indicators were considered lowly variable with the coefficient of variation CV < 20%, moderately variable with CV = 20–40%, and highly variable with CV > 40% (Butnik 1987).

Table 1. Phytocoenotic characteristics of *Rhodiola rosea* coenopopulations in the Altai Mountains

CP	Location, height above sea level	Habitat / dominant species	TPC, %
1	Chemalsky district, Iolgo ridge. The right side of the valley near Karakol lakes, 1840 m	**Subalpine forbs among cedar woodlands on a slope** *Rhodiola rosea, Carex aterrima, Bistorta officinalis, Stellaria bungeana, Saussurea latifolia, Alchemilla vulgaris, Swertia obtusa*	85
2	The same location. Left side of the valley near Karakol lakes, 1920 m	**Grassy tundra along the edge of dwarf birch on a leveled area in the upper part of the slope** *Rhodiola rosea, Carex sempervirens, Bistorta elliptica, Allium schoenoprasum, Saussurea alpina, Gentiana algida*	95
3	Kosh-Agachsky district, Kurai ridge. Near Kurai village, 2715 m	**Alpine meadow on a slope along a stream** *Anthoxanthum alpinum, Trisetum mongolicum, Festuca kryloviana, Poa altaica, Carex aterrima, Aconitum altaicum, Dracocephalum peregrinum, Bistorta officinalis, Oxyria digyna, Allium schoenoprasum, Aster alpinus, Rhodiola rosea*	80–90
4	Kosh-Agachsky district, Northern Chuysky ridge. Steep left side of the Aktru river valley, 2385 m	**Subalpine forbs among the thickets of willows in the hollow of the drain** *Salix cinerea, Geranium albiflorum, Rhodiola rosea, Saussurea frolowii, Aegopodium alpestre, Aquilegia sibirica, Hedysarum neglectum*	70
6	Kosh-Agachsky district, near Chagan-Uzun village. Mount Sukor, 2828 m	**Stony-rubbly tundra on a steep slope in the hollow of the drain** *Lagotis integrifolia, Minuartia verna, Dracocephalum nutans, Cerastium lithospermifolium, Rhodiola rosea*	15
8	Kosh-Agachsky district, Southern Chuysky ridge, Akkol river valley, 2453 m	**Unformed vegetation on the pebbles along the river bank** *Trisetum mongolicum, Festuca altaica, Festuca brachyphylla, Arctopoa tibetica, Archangelica decurrens, Tripleurospermum ambiguum, Rhodiola rosea*	15–20

CP	Location, height above sea level	Habitat / dominant species	TPC, %
9	The same location, the mouth of the Verkhniy Turaoyuk river (the left tributary of the Akkol), 2324 m	**Bushes along the river** *Salix pentandra, Salix sajanensis, Salix glauca, Spiraea alpina, Lonicera altaica, Arctopoa tibetica, Rheum compactum, Archangelica decurrens, Primula nivalis, Rhodiola rosea, Rhodiola algida*	70–90

Note: CP – coenopopulation; TPC – total plants cover.

The indicators from different coenopopulations were compared by the one-way ANOVA to determine the statistical significance of the differences (Zhukova 2020) using Statistica 8.0 (2008). Statistically significant differences were determined at $p < 0.05$.

To determine the correlation between different indicators, the Spearman's correlation coefficient (rs) was calculated based on the average values of characteristics in coenopopulations. The correlation was considered distinct at $0.5 < r_s \geq 0.7$, high at $0.7 < r_s \geq 0.9$, and strong at $r_s > 0.9$ (Zhukova 2021).

Result

Rhodiola rosea leaves are fleshy, sessile and alternate. The leaves are arranged perpendicular to the stem or upward at an angle to the stem. The leaf shape varies from the base of the stem to its upper part: from obovate to lanceolate and oblong; the leaf margin also varies from almost entire to dentate. The leaf length in the studied coenopopulations varies from 20.3 to 34.4 mm; the leaf width ranges from 8.4 to 11.7 mm. It was also previously found that the leaf size is different in male and female plants (Prokopyev et al. 2021).

As was shown earlier, female shoots have 16% more leaves, but the ratio of leaf number to shoot length (an integral characteristic of shoot leafiness) is 18% less, which indicates longer internodes. The length, width and dry weight of leaves in female shoots are less compared to those in male shoots, but the differences are not statistically significant (Prokopyev et al. 2021).

Rhodiola rosea leaves exhibit dorsoventral anatomy. The palisade mesophyll contains mainly 2 densely spaced cells, yet up to 4 layers were found in plants growing under different conditions. Leaves are amphistomatous; stomata are predominantly anisocytic and present on both leaf sides. Most researchers report a similar description of *Rh. rosea* stomata (Kim 1999; Pautov and Pautova 2003; Golovko et al. 2007; Shmakova and Ermolaeva 2021). The epidermis is single-layered; the cell shape is round, slightly wavy or wavy, and is different depending on the coenopopulation.

In total, 22 characteristics of the anatomical structure of the *Rh. rosea* leaf were analyzed. Within one geographic location, the specimens showed fewer statistically significant differences compared to specimens from different areas. For example, for leaves of plants from the Akkol mountain-glacial basin (CP 8 and CP 9), only one characteristic (leaf thickness) with a significant difference (4.5% of the total number of characteristics) was found; the leaves of plants from the Iolgo ridge (CP 1 and CP 2) exhibited 8 characteristics (36.4%). In specimens from different geographical locations, the maximum number of significant differences in characteristics attained 15 (68.2%) for compared pairs CP 2–CP 4 and CP 2–CP 6, 13 differences (59.1%) for pairs CP 3, ♀–CP 2, 11 differences (50%) for pairs CP 3, ♂–CP 2 and CP 2–CP 8, and 10 differences (45.5%) for pairs CP 2–CP 9, CP 4–CP 6, and CP 6–CP 8. It is obvious that the leaves of CP 2 specimen are distinctly different not only from the leaves of specimens from other locations, but also for specimens within one geographic location.

Among the studied specimens, the number of cells of the upper epidermis is maximum (accordingly, the size of the upper epidermis cells is minimal) in CP 2 specimen (739.8 pcs per 1 mm^2, 1958.8 μm^2 cell size); large number of cells and low cell sizes were also found for CP 1 (537.0 pcs per 1 mm^2, 2276.3 μm^2 cell size), CP 3, ♂ (529.3 pcs per 1 mm^2, 2586.2 μm^2 cell size), and CP 3, ♀ (498.6 pcs per 1 mm^2, 2998.7 μm^2 cell size), without statistically significant differences; CP 6 (350.7 pcs per 1 mm^2, 4794.0 μm^2 cell size) and CP 9 (395.5 pcs per 1 mm^2, 4804.0 μm^2 cell size) differ in the smallest cell number and the largest cell size (Suppl. material 1: Table 2, Fig. 2). CP 2 specimen is characterized by the maximum number of stomata on the upper epidermis (109.8 pcs), which is statistically different from other coenopopulations. The minimum number of stomata on the upper epidermis was found in CP 6 specimen (48.6 pcs), but this specimen does not show significant differences from CP 3, ♀ and CP 9 specimens. In this case, a relative value such as the stomatal index of the upper epidermis does not indicate statistically significant differences in all coenopopulations and averages from 11.3 to 13.8%; this indicator is predominantly low-variable; only in CP 6 and CP 9 specimens, the coefficient of variation is moderately variable and amounts to 25.7 and 23.8%, respectively. In plants growing in coenopopulations with insufficient water supply (CP 1 and CP 2), the number of stomata on the upper epidermis is higher compared to plants growing under sufficient and excess water supply. E.F. Kim (1999) reported the same trend when studying the physiological features of *Rh. rosea* in response to different water regimes.

The number of cells of the upper epidermis in CP 1 and CP 3 specimens (with no statistically significant differences) is comparable with the data obtained for *Rh. rosea* species from St. Petersburg (on average 561.6 pcs). The coefficient of variation for plants from the Altai Mountains (10.5–19.4%) was lower than that for plants introduced at V.L. Komarov Botanical Institute RAS (on average 23.2%) (Pautov and Pautova 2003).

The obtained data on the number of stomata are comparable with the data for *Rh. rosea* introduced in Murmansk region (123 pcs on the upper epidermis and 95 pcs on the lower one) (Shmakova and Ermolaeva 2021).

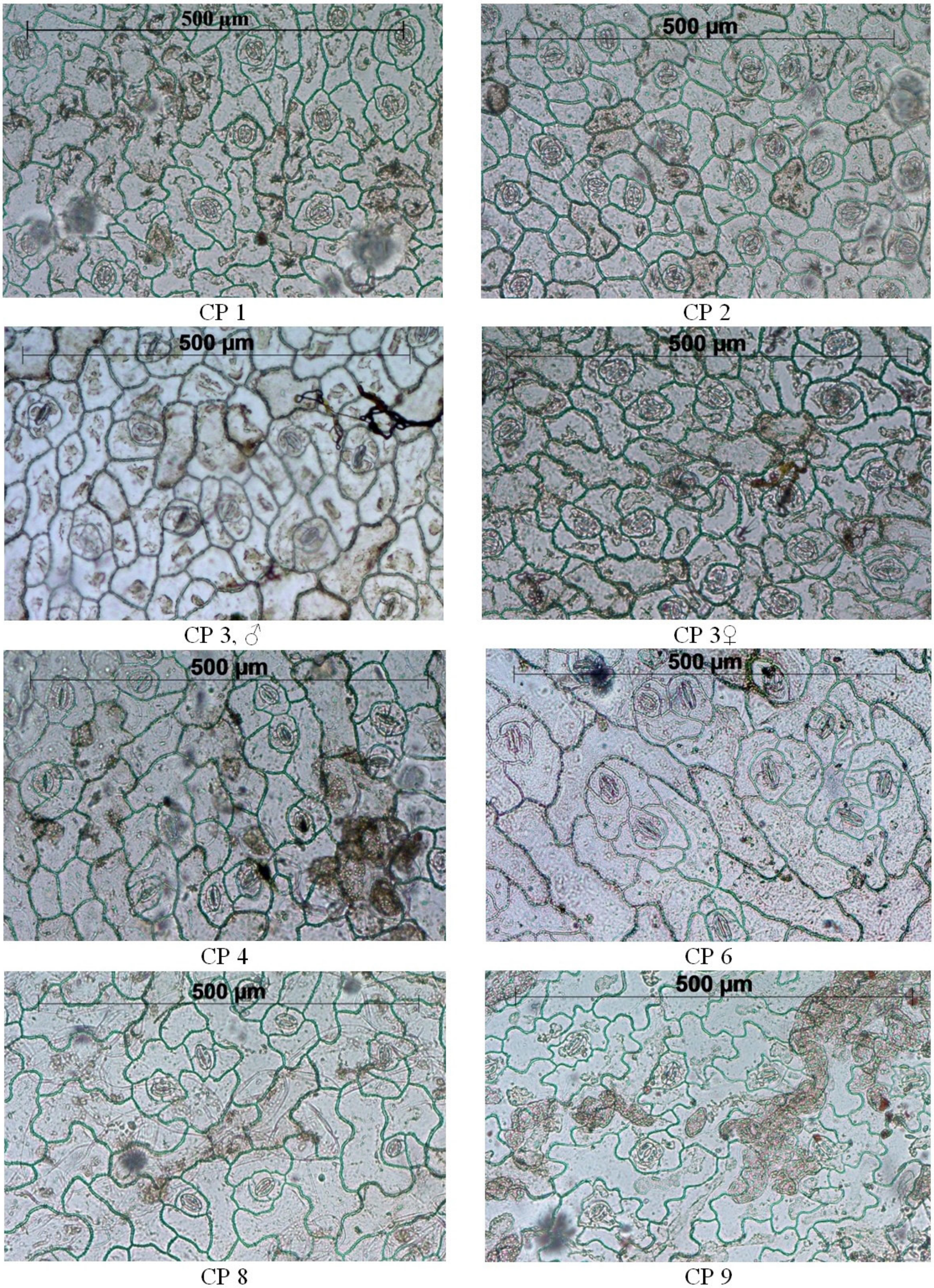

Figure 2. Upper epidermis of leaves of *Rh. rosea* from different coenopopulations.

The sizes of the upper and lower epidermis stomata within the same coenopopulation are virtually similar; for example, in CP 3 specimen, ♂, the stomata on the upper and lower epidermis are 27.1 µm long and 18.7 µm wide; the coefficient of variation for these characteristics does not exceed 9.5%. Almost complete coincidence in sizes of the upper and lower epidermis stomata can be observed in CP 8 specimen. The largest stomata are found in CP 6 specimen (32.1 µm long, 18.6 µm wide on the adaxial side, and 30.5 µm long, 19.0 µm wide on the abaxial side). The stomatal size is the least variable characteristic, the coefficient of variation ranges from 5.5 to 14.2%.

The number of cells and stomata on the lower and upper epidermis is maximum in CP 1 and CP 2 specimens from the Iolgo ridge (on average 814.1–854.4 cells and 125.8–137.6 stomata); cell sizes are minimal (on average 2052.1–2267.8) (Suppl. material 1: Table 2, Fig. 3). The small-celled epidermis with a large number of small stomata is an adaptive response of these plants to water deficit and increased insolation, which was reported by N.Y. Shmakova and O.V. Ermolaeva (2021). The number of cells and stomata in other specimens are not significantly different; however, it should be noted that the largest cells of the lower epidermis (as well as the upper one) were found for plants from the Akkol mountain-glacial basin (on average up to 5566.8 µm2), which is an adaptive response to shading and good water supply.

A greater leaf and mesophyll thickness was observed in CP 6 specimen (629.4 and 570.5 µm, respectively) and in CP 3 specimen from the Kurai ridge (on average 540.0–557.1 and 461.3–495.5 µm, respectively). A similar pattern was revealed for palisade and spongy mesophyll. The thinnest leaves and less developed mesophyll (including palisade and spongy) were characteristic of CP 1 specimen and CP 8 and CP 9 specimen from the Akkol mountain-glacial basin (Suppl. material 1: Table 2, Fig. 4). The differences in the thickness of the upper and lower epidermis are not statistically significant, but the average thickness of the lower epidermis is slightly greater (by 0.1–3.0 µm, which is 0.3–10.7%). T.K. Golovko et al. (2007) also reported a thicker lower epidermis, but indicated a difference of 10–20%. Palisade mesophyll consists of two layers of cells, which was also reported in (Golovko et al. 2007; Shmakova and Ermolaeva 2021). The ratio of the sizes of palisade and spongy mesophyll is almost similar for all specimens and does not show statistically significant differences, similar to the cell width in the most active photosynthetic layer.

CP 6 specimen exhibits a greater development in the most active photosynthetic layer. This specimen also shows the smallest development of the vascular bundle (11185.1 μm^2), which indicates the plant adaptive response to good light conditions and insufficient or unstable water supply. The sizes of the upper layer of columnar mesophyll in CP 6 specimen are similar to those in *Rh. rosea* from the Polar-Alpine Botanical Garden, Murmansk region. According to N.Y. Shmakova and O.V. Ermolaeva (2021), the cell length and width are 81.2 µm and 50.2 µm, respectively, and the coefficient of variation is 16.8–25.1%; according to our data, the length and width are 85.0 µm and 53.5 µm, respectively, and the coefficient of variation attains 16.8–22.9%.

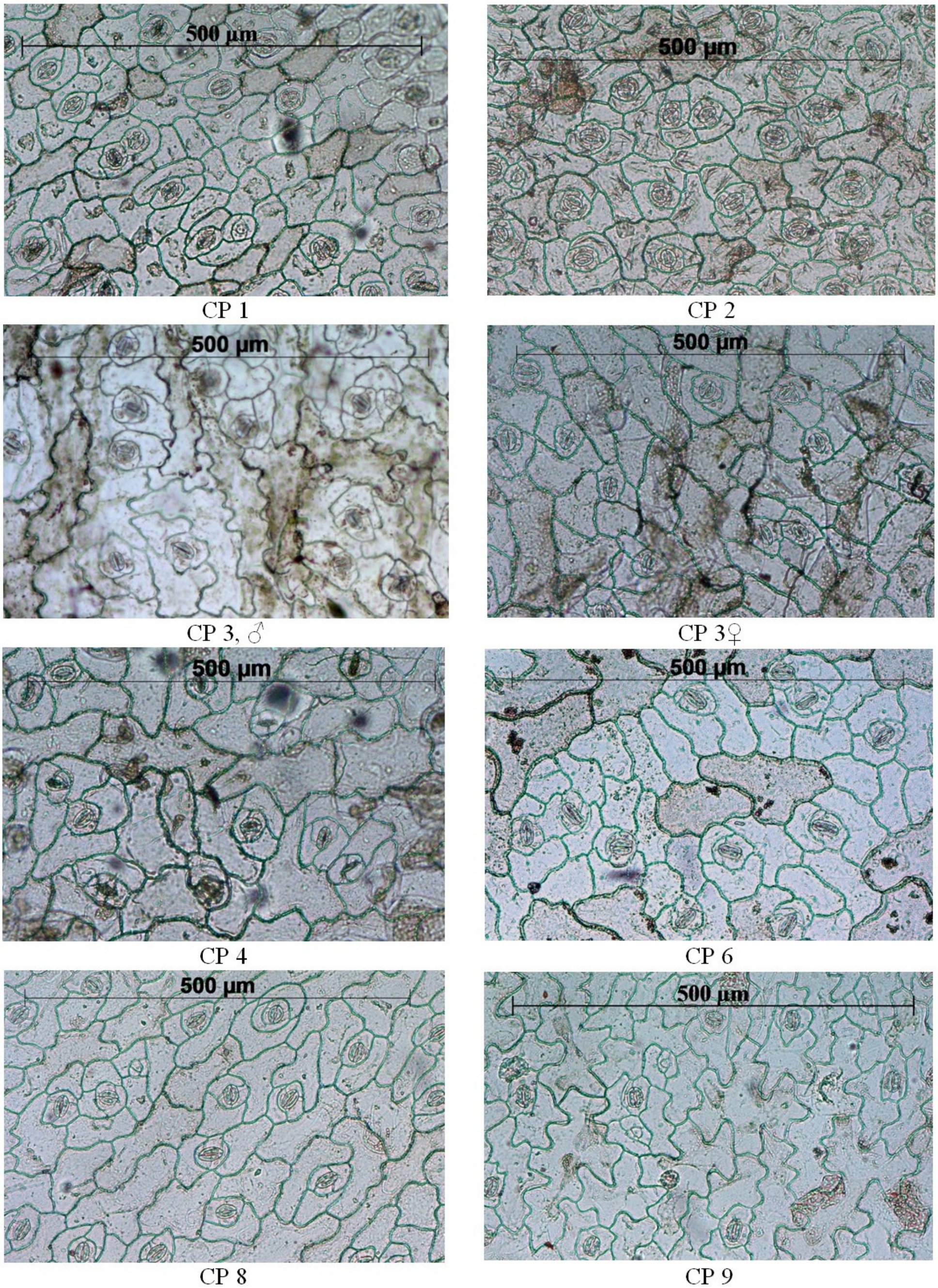

Figure 3. Lower epidermis of leaves of *Rh. rosea* from different coenopopulations.

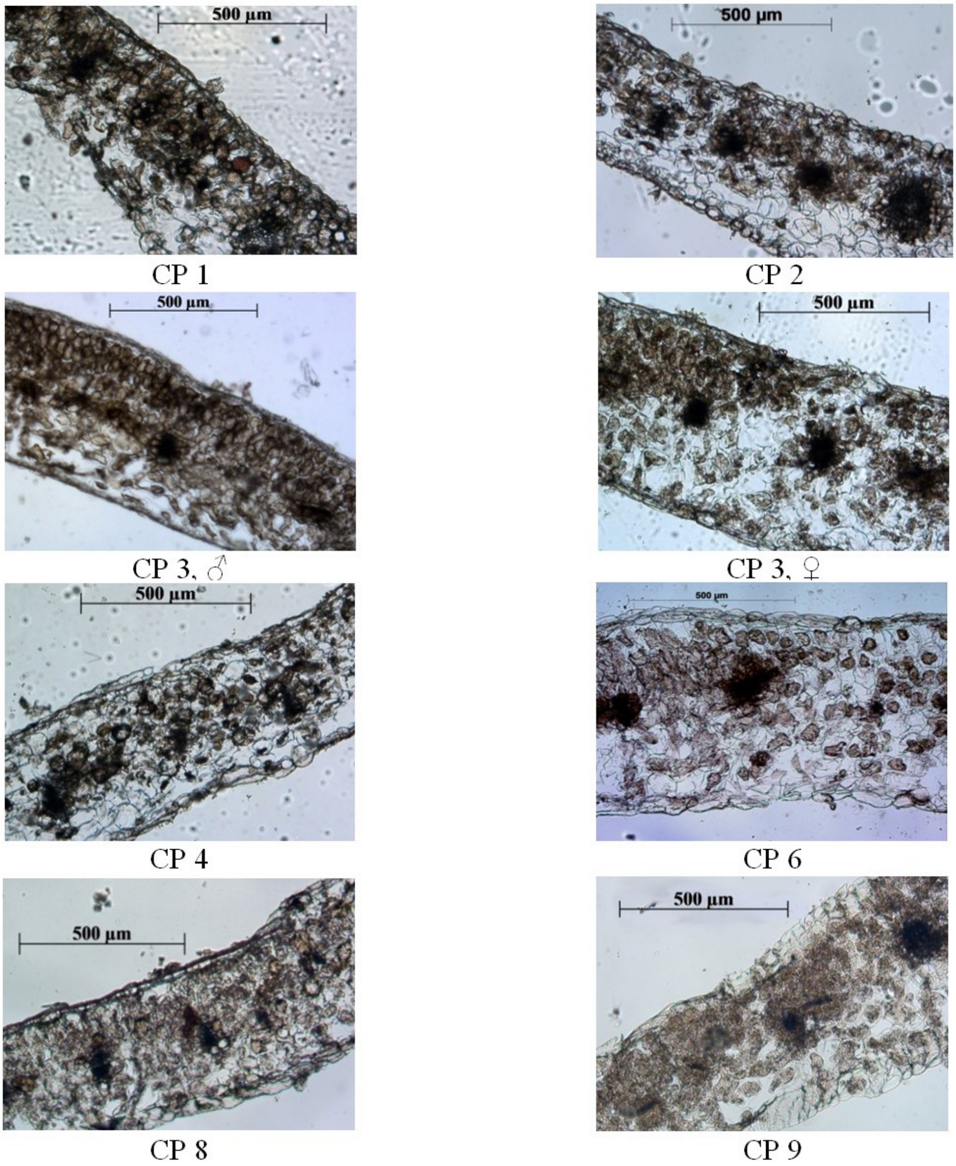

Figure 4. Cross sections of leaves of *Rh. rosea* from different coenopopulations.

The characteristics of the leaf cross section are of a low and medium level of variation.

Comparison of male and female plants of *Rh. rosea* from the Kurai ridge (CP 3) revealed statistically significant differences only for 3 characteristics – the thickness of the lower epidermis, the length of the cells of the upper mesophyll layer, and the size of vascular bundles, which were found large in female plants. The sizes of the lower epidermis stomata are almost similar (length and width are 27.1 and 18.7 μm, respectively, in the male plant, and 27.5 and 18.9 μm, respectively, in the female plant). Leaf mesophyll in the female plant is less developed; yet, the thickness of the spongy and palisade mesophyll and their ratio are not statistically different (Suppl. material 1: Table 2). In this case, the cells of the most photosynthetically active upper mesophyll layer in the male plant are smaller than those in the female plant. The female plant exhibits larger vascular bundles and more developed vascular system.

Thus, the specimen from the Iolgo ridge (CP 2) has a small-celled epidermis with a large number of small stomata, which indicates its adaptive response to good light conditions. Plants in CP 1 grow under worse light conditions. The epidermis of CP 9 specimen consists of a smaller number of large cells, and the thickness of the leaf and mesophyll is relatively smaller, which indicates worse light conditions. CP 8 specimen is characterized by more pronounced heliophytic features. CP 6 specimen shows minimal development of vascular bundles, which indicates periodical wetting-drying conditions.

Generative shoots of female plants from the Kurai ridge (CP 3) are adapted to long-term functioning: their vascular system is developed for a greater water inflow and nutrient outflow. In the female plant, the most photosynthetically active layer of cells is more developed, which promotes a more active production process in its leaves. At the same time, the leaves of female plants are characterized by longer internodes greater due to light exposure, which results in decreased thickness of the leaf, mesophyll and its layers; sufficient water supply to the leaf through the developed vascular system makes the cells of the upper layer of palisade mesophyll larger than those in the male plant due to decreased xerophytization.

The difference in the altitude between the lowest growing CP 1 (1840 m) and the highest growing CP 6 (2828 m) is about 1 km. In (Prokopyev et al. 2021), we showed that leaf dimensions (length, width, area and thickness) do not significantly correlate with the leaf dry weight and its specific surface density that indicates the accumulation of dry matter per unit leaf area. However, the leaf length is negatively correlated with the altitude (r_s = –0.76) and leaf thickness (r_s = –0.80), while the leaf thickness is positively correlated with the altitude (r_s = 0.88). That is, the leaves become shorter and thicker at higher altitudes.

This study revealed some correlation between anatomical characteristics of Rh. rosea leaves and the altitude of the coenopopulation habitat. As the altitude increases, the epidermis thickness on the adaxial side of the leaf grows (r_s = 0.76), and the stomata length increases on both the adaxial (r_s = 0.70) and abaxial (r_s = 0.84) sides, but the number of epidermal cells per unit area decreases on the adaxial (r_s = –0.70) and abaxial (r_s = –0.89) sides, and the number of stomata per unit leaf area on the adaxial (r_s = –0.72) and abaxial (r_s = –0.92) sides decreases as well. The sizes of cells in the lower epidermis show high negative correlations with the number of cells (r_s = –0.82) and stomata (r_s = –0.79), which is quite logical.

The stomatal index of the upper epidermis is positively correlated with the stomatal index of the lower epidermis (r_s = 0.75) and does not significantly correlate with other anatomical and morphological characteristics of the leaf; apparently, the index changes synchronously on both leaf sides.

At higher altitudes, an increase in the leaf thickness is accompanied by the increased total thickness of photoassimilating tissue ($r_s = 0.85$): the thickness of both palisade ($r_s = 0.82$) and spongy ($r_s = 0.80$) mesophyll increases. An increase in the thickness of palisade mesophyll is due to the increased length of its cells ($r_s = 0.85$).

The area of vascular and mechanical tissues in the leaf vein were not measured in the study. The vein area shows high positive correlation only with the leaf area ($r_s = 0.71$) and dry mass ($r_s = 0.81$), and the leaf area, in turn, is positively correlated with the leaf length ($r_s = 0.81$). Since the vein consists of vascular and mechanical tissues, it could be assumed that the vein increases due to the increased area of vascular tissue as the thickness of photoassimilating tissue increases, but there is no such correlation; therefore, the increased vein associated with the increased leaf area is due to the increased area of mechanical tissue of the vein.

Apparently, the established correlations for anatomical and morphological characteristics are adaptive responses to increased insolation and decreased partial pressure of carbon dioxide at higher altitudes. The correlation patterns can be described as follows: the higher *Rh. rosea* grows in the mountains, the shorter and thicker its leaves become, the thickness of integumentary tissue with cuticle increases, the number of stomata and cells on integumentary tissue per epidermis unit area decreases (cell sizes increase), and the thickness of photoassimilating tissue grows – both palisade and spongy mesophyll (cell length in palisade mesophyll). Increased thickness of spongy mesophyll is probably associated with decreased partial pressure of carbon dioxide at high altitudes. Increased thickness of spongy mesophyll with loosely arranged cells with intercellular spaces increasing CO_2 diffusion improves gas exchange and accumulation of CO_2 in leaf tissues.

Conclusions

Rhodiola rosea has a reserve of adaptive plasticity, which allows the species to grow under different light and water conditions. Depending on the coenopopulation, the studied plants exhibited different degrees of heliophytic and xerophytic adaptations. Comparison of male and female plants of *Rh. rosea* growing in the Kurai ridge (CP 3) showed that the photosynthetically active layer and vascular tissue are better developed in leaves of female plants.

It was established that *Rh. rosea* growing in the mountains bear shorter and thicker leaves; the thickness of integumentary tissue with cuticle increases at higher altitudes, the number of stomata and cells on integumentary tissue per epidermis unit area decreases, and the thickness of photoassimilating tissue grows. Changes in anatomical and morphological characteristics of the plants are adaptive responses to increased insolation at higher altitudes and, probably, to decreased partial pressure of carbon dioxide.

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References

Baranova MA (1985) Classification of morphological types of stomata. Botanical Journal 70(12): 1585–1595. [In Russian]

Belenovskaya LM, Medvedeva LI (2009) Sem. Crassulaceae DC. – Crassulaceae. In: Budantsev AL (Ed.) Plant resources of Russia: Wild flowering plants, their component composition and biological activity. T. 2. Partnership of scientific publications KMK, St. Petersburg–Moscow, 160–174. http://publication.pravo.gov.ru/document/0001202307210008?index=27 [In Russian]

Bezdelev AB, Bezdeleva TA (2006) Life forms of seed plants of the Russian Far East. Dalnauka, Vladivostok, 296 pp. [In Russian]

Blinova IV (2014) Ecological-coenotic and ontogenetic strategies of rare species of vascular plants of the Murmansk region. Bulletin of the Kola Scientific Center of the Russian Academy of Sciences 4(19): 83–95. [In Russian]

Boardman NK (1977) Comparative Photosynthesis of Sun and Shade Plants. Annual Review of Plant Physiology 28: 355–377. https://doi.org/10.1146/annurev.pp.28.060177.002035

Butnik AA, Timchenko OV (1987) Structure of the epidermis of leaves of species of the family Chenopodiaceae. Botanical Journal 72(8): 1021–1030. [In Russian]

Chadaeva VA, Shhagapsoev SH (2016) Analysis of survival strategies of species *Allium* L. in the Russian Caucasus. South of Russia: ecology, development 11(1): 104–118. https://doi.org/10.18470/1992-1098-2016-1-104-118 [In Russian]

Dorogina OV, Kuban IN, Achimova AA, Williams N, Lashchinskiy NN, Zhmud EV (2023) Morphometric characteristics and genetic ISSR marker variability in *Rhodiola rosea* L. (Crassulaceae) in different ecological and geographic conditions in the Altai Republic. International Journal of Molecular Sciences 24(20): 15224. https://doi.org/10.3390/ijms242015224

Ezau K (1980a) Anatomy of seed plants: in 2 books. Book 1. Mir, Moscow, 224 pp. [In Russian]

Ezau K (1980b) Anatomy of seed plants: in 2 books. Book 2. Mir, Moscow, 560 pp. [In Russian]

Frolov YM, Poletaeva II (1998) *Rhodiola rosea* in the European Northeast. Ural Branch of the Russian Academy of Sciences, Ekaterinburg, 192 pp. [In Russian]

Givnish TJ (1988) Adaptation to Sun and Shade: A Whole-plant Perspective. Australian Journal of Plant Physiology 15: 63–92.

Golovko TK, Dalke IV, Bocharov DS, Babak TV, Zakhozhiy IG (2007) Crassulaceae in cold climates (biology, ecology, physiology). Nauka, St. Petersburg, 205 pp. [In Russian]

Goncharova SB (2006) Sedumaceae (Sedoideae, Crassulaceae) of the flora of the Russian Far East. Dalnauka, Vladivostok, 223 pp. [In Russian]

Goryshina TK (1979) Plant ecology. Higher School, Moscow, 368 pp. [In Russian]

Ishmuratova MM (2006) Rhodiola Iremelskaya in the Southern Urals. Nauka, Moscow, 252 pp. [In Russian]

Ishmuratova MM (Ed.) (2020) Methodology for studying populations of rare and resource plant species in protected natural areas of the Republic of Bashkortostan. Bashkir encyclopedia, Ufa, 276 pp. [In Russian]

Ivanova LA (2014) Adaptive features of leaf structure in plants of different ecological groups. Russian Journal of Ecology 45(2): 107–115. https://doi.org/10.7868/S0367059714020024

Kästner A (1972) Blattepidermis-Strukturen bei Carlina. Flora 161(3): 225–255. [In German]

Kim EF (1999) *Rhodiola rosea* (golden root) and the biological basis for its introduction into culture. Altai State University Publishing House, Barnaul, 176 pp. [In Russian]

Kopanina AV, Vlasova II, Vacerionova EO (2017) Structural adaptation of woody plants to volcanic landscapes of the Kuril Islands. Vestnik of Far Eastern Branch of Russian Academy of Sciences 1: 88–96. [In Russian]

Kozhevnikov YP (2007) Plants of Central Asia: Based on materials from the Komarov Botanical Institute of the Russian Academy of Sciences. Vol. 16: Crassulaceae–Saxifragaceae. Scientific Society KMK publications, Moscow, 135 pp. [In Russian]

Kozlov AA (2023) On approval of the List of flora objects included in the Red Book of the Russian Federation. Order No. 320 of 23.05.2023. Ministry of Natural Resources and Ecology of the Russian Federation, Moscow, 27 pp. [In Russian]

Krasnov EA, Saratikov AS, Surov YP (1979) Plants of the Crassula family. Tomsk University Publishing House, Tomsk, 208 pp. [In Russian]

Legoshchina OM, Neverova OA, Bykov AA (2013) The variability of the anatomical structure of pine needles of *Picea obovata* Ledeb. Under the effect of emissions from the industrial zone of Kemerovo. Contemporary Problems of Ecology 20(5): 733–739. [In Russian]

Maftei D-I, Maftei D-E (2015) Several histo-anatomical aspects on the rhizome structure in *Rhodiola rosea* L. Scientific Study & Research – Biology 24(1): 109–111.

Maftei D-I, Maftei D-E (2018) Preliminary histo-anatomical research on *Rhodiola rosea* L. in conventional and in vitro cultures. Scientific Study & Research – Biology 27(1): 58–62.

Maftei D-I, Maftei D-E (2019) A comparative morpho-anatomical research in *Rhodiola rosea* L. (golden root) in conventional and in vitro cultures. Scientific Study & Research – Biology 28(1): 94–100.

Magomedmirzaev MM, Guseinova ZA, Alibegova AN, Magomedova SM (2013) The problem of plant adaptive strategies. Publishing house "Science DNTs", Makhachkala, 300 pp. [In Russian]

Nekratova NA, Nekratov NF (2005) Medicinal plants of the Altai-Sayan mountain region. Resources, ecology, coenological complexes, population biology, rational use. Tomsk State University Publishing House, Tomsk, 228 pp. [In Russian]

Nukhimovsky EL (1997) Fundamentals of biomorphology of seed plants. Biomorph organization theory. T. 1. Nedra, Moscow, 630 pp. [In Russian]

Oleinikova EM (2016) Realization of adaptive potential in plants (using the example of tap-root grasses). Modern problems of science and education 1: 57. https://science-education.ru/ru/article/view?id=24104 [In Russian]

Pautov AA (2012) Morphology and anatomy of vegetative organs of plants: textbook. St. Petersburg University Publishing House, St. Petersburg, 336 pp. [In Russian]

Pautov AA (Ed) (2003) Questions of comparative and ecological anatomy of plants. St. Petersburg University Publishing House, St. Petersburg, 220 pp. [In Russian]

Pautov AA, Pautova IA (2003) Intrageneric relationships of representatives of *Rhodiola* L. (Crassulaceae DC.) according to data on leaf morphology and epidermal structure. Bulletin of St. Petersburg University. Ser. 3 3 (1): 13–19. [In Russian]

Peshkova GA (1994) Family Crassulaceae – Crassulaceae. In: Malyshev LI, Peschkova GA (Eds) Flora of Siberia. Vol. 7. Science, Novosibirsk, 152–168 pp. [In Russian]

Poliakova TA (2022) Adaptive strategies and genetic stability of species of the genus *Spiraea* (Rosaceae) in natural population systems. Advances in Current Biology 142(5): 530–542. https://doi.org/10.31857/S0042132422050118 [In Russian]

Polozhiy AV, Revyakina NV, Kim EF, Sviridova TP (1985) Rhodiola rosea, golden root – *Rhodiola rosea* L. In: Sobolevskaya KA (Ed) Biology of Siberian plants in need of protection. Science, Novosibirsk, 85–114. [In Russian]

Popova OA (2011) Adaptation characteristics of prevernal early-вlooming plants of Eastern Zabaikalye. Scientific notes of the Transbaikal State Humanitarian and Pedagogical University 1(36): 102–110.

Prokopyev AS, Yamburov MS, Chernova OD, Kataeva TN, Prokopyeva ES, Machkinis EY, Kuznetsov AA (2021) Ecological and morphological features of *Rhodiola rosea* L. in natural populations in the Altai Mountains. Acta Biologica Sibirica 7: 529–544. https://abs.pensoft.net/article/78936/

Prokopyev AS, Yamburov MS, Kataeva TN, Chernova OD, Prokopyeva ES (2023) Reproductive biology of *Rhodiola rosea* in natural conditions of the Altai Mountains. Bulletin of Tomsk State University. Biology 64(4): 52–80. https://doi.org/10.17223/19988591/64/3 [In Russian]

Satsyperova IF, Kurkin VA, Zapesochnaya VG, Pautova IA (1991) Chemical composition of rhizomes of *Rhodiola arctica* Boriss., introduced to the Leningrad region. Plant Resources 27(4): 55–60. [In Russian]

Semenova GP (2007) Rare and endangered flora species of Siberia: biology and protection. Academic publishing house "Geo", Novosibirsk, 408 p. [In Russian]

Shmakova NY, Ermolaeva OV (2021) Anatomical and physiological characteristics of *Rhodiola rosea* L. in the conditions of culture of the Polar-Alpine Botanical Garden (Murmansk region). Subtropical and ornamental gardening 77: 141–151. https://doi.org/10.31360/2225-3068-2021-77-141-150 [In Russian]

Vehov VN, Lotova LI, Filin VR (1980) Workshop on the anatomy and morphology of higher plants. Moscow State University Publishing House, Moscow, 196 pp. [In Russian]

Zakharevich SF (1954) On the method of describing the epidermis of a leaf. Bulletin of Leningrad University 4: 65–75. [In Russian]
Zhukova AA, Minets ML (2020) Biometrics: manual. In 3 hours. Part 2. Basic data analysis techniques. BSU, Minsk, 151 pp. [In Russian]
Zhukova AA, Minets ML (2021) Biometrics: manual. In 3 hours. Part 3. Correlation and Regression. BSU, Minsk, 103 pp. [In Russian]
Zhumagul MZh, Kurmantayeva AA, Hohn M (2020) Features of the anatomic structure of the medicinal plant *Rhodiola rosea* L. in East Kazakhstan. Experimental Biology 4(85): 01. https://doi.org/10.26577/eb.2020.v85.i4.01 [In Kazakh]
Zlobin YuA, Sklyar VG, Klimenko AA (2013) Populations of rare plant species: theoretical foundations and study methods. University Book, Sumy, 439 pp. [In Russian]

Supplementary material 1

Table 2. Anatomical characteristics of leaves of *Rh. rosea* from different coenopopulations

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