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RESEARCH ARTICLE

Anatomy of the leaf blades of *Waldsteinia ternata* (Stephan) Fritsch (Rosaceae) grown under different light conditions

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Abstract

The paper reports the results of a comparative study of the anatomical structures of the leaf blades of *Waldsteinia ternata* grown under different light conditions in the Siberian Botanical Garden of Tomsk State University. *Waldsteinia ternata* is a tertiary nemoral relict from the mountains of southern Siberia, which is found in a limited number of taiga communities due to narrow environmental tolerance to various factors. The species remains poorly studied; comprehensive studies of the anatomical features of its leaves have not been performed on the territory of Russia. Leaves of *W. ternata* are dorsoventral and amphistomatous with anomocytic type stomata. The plants are classified as mesophytes. The relationship between the development of the anatomical structure of leaves and light conditions was revealed. The *W. ternata* plants grown in the sun showed an increased number of stomata and epidermal cells, an increased thickness of the leaf and mesophyll, and an increased number of cells of the upper and lower epidermis, that is, the plants exhibited heliophytic features of plant adaptation to good light conditions. At the same time, the vascular tissues of the plants grown in the sun were less developed, which reflected their adaptation to unfavorable water conditions. A number of relative indicators, such as the stomatal index of the lower epidermis, the ratio of the palisade to spongy mesophyll, and the ratio of xylem to phloem, did not change under different growth conditions. Thus, under different light and water conditions, *W. ternata* acquires helioxeromorphic or sciomesomorphic features.

Keywords

Adaptive variability, epidermis, mesophyll, plant ecology, stomatal index, Western Siberia

Introduction

Conservation of plant species as a source of biological diversity and genetic resources is an essential part of the Global Strategy for Plant Conservation adopted in 2002 at the VI International Conference of the Parties to the Convention on Biological Diversity (Jackson 2012). Russian botanical gardens are currently included in the world's largest plant conservation network BGCI (Botanic Gardens Conservation International) (Gorbunov and Demidov 2012), and they are becoming increasingly important for ex situ plant protection. Botanical collections of living plants are used by scientists to conduct in-depth studies of the biology of rare species, assess their prospects for introduction, and use introduced plants as donors for subsequent reintroduction into natural habitats. Relict species that have been preserved since ancient times are of particular interest among the plants of the natural flora of Siberia that need conservation.

Waldsteinia ternata (Steph.) Fritsch (Rosaceae) is a Eurasian species with a disjunctive range and classical location in the Khamar-Daban ridge (Chepinoga et al. 2019). It grows in dark coniferous, larch, poplar, birch, and mixed forests, river floodplains, bushes, and willow woods (Yamskikh 2015). In the Khamar-Daban ridge, it is often one of the codominants in the herb-shrub layer in floodplain forests. The densest populations are observed on forest fringes and along forest paths (Kazanovskiy and Chepinoga 2020).

Based on the data of molecular genetic studies, the modern phylogenetic classification assigns a small genus *Waldsteinia* Willd. to the genus *Geum* L. (Potter et al. 2007). In this study, we adhere to the previous taxonomic concept and consider this species in the rank of *W. ternata* since the taxonomy of species of the genus is not sufficiently complete.

Waldsteinia ternata is of undoubted scientific interest since it is a tertiary nemoral relict from the mountains of southern Siberia, as well as a rare plant listed in the Red Data Books of Krasnoyarsk Territory (Stepanov 2012), Republic of Buryatia (Krasnopevtseva and Krasnopevtseva 2013), Republic of Khakassia (Martynova 2012), and Irkutsk Region (Kazanovskiy and Chepinoga 2020), which provoked studies of natural populations of the species. For instance, A.N. Lisina (2012) found that the maximum sizes of the vegetative organs of *W. ternata* from the mountains of the Western Sayan and Khamar-Daban were observed in plants growing in mixed cedar forests, and the maximum sizes of generative ones were observed in plants growing in pine-birch forests under better light conditions. I.E. Yamskikh (2015) classifies *W. ternata* as a species sensitive to heat, moisture, soil conditions, violents, and phytocenotic patients, which are found in a limited number of taiga communities in the mountains of southern Siberia due to narrow environmental tolerance to various factors. She considers this species to be a pacific relic, a closely related species of which grow in the mountain forests of Southeast Asia, and a relict-degradant.

Currently, *W. ternata* is grown in various botanical gardens in Russia as a promising ground-cover stolon-forming spring-summer-winter-green herbaceous ornamental plant; however, in general, the plant remains poorly studied. The biology of the species in the introduction was studied in most detail by G.P. Semenova (2007), who classifies it as a vegetatively mobile plant that is promising for introduction in Western Siberia. Comprehensive studies of the anatomical features of the species have not been performed on the territory of Russia.

The aim of this study is a comparative investigation of the anatomical structures of the leaf blades of *W. ternata* introduced under different light conditions in the Siberian Botanical Garden of Tomsk State University.

The conducted anatomical studies will add to ecological characteristics of the species, assess the plasticity and potential of the species, and determine the most optimal growth conditions in the introduction. This research is important for the conservation and protection of *Waldsteinia ternata* in a changing climate.

Material and methods

Plants of *Waldsteinia ternata* were collected in the Botanical Garden of Irkutsk State University in 2008. Specimens for the study were grown in the collection-experimental site of the Siberian Botanical Garden of Tomsk State University in the sun and in the shade. The authors used generally accepted methods, publications of A.A. Pautov (2003, 2012), R.P. Barykina et al. (2004) as the methodology to study leaf anatomy.

For the study, we used leaves without visible damage collected from five generative plants for each growing condition. Leaf sections were prepared from 5 leaves collected from 5 plants, so the authors analyzed at least 25 sections for each variant of the experiment.

Temporary specimens of fresh leaves were cut via the freezing microtome MZ–2. Cross sections were taken from the middle part of the leaf. The section thickness was 30–60 μm. Epiderm was cut with a razor in the middle third of the blade between the leaf margin and the midrib.

The photos of leaf microsamples and microscopic measurements were made using the Carl Zeiss Axio Lab. A1 light microscope (Germany) with the AxioCam ERc 5s digital camera by means of the Axio Vision 4.8 software. For each characteristic, 25 values were analyzed.

The measurement results were statistically processed with the Statistica 8.0 software. The authors determined the following parameters: M – arithmetic mean, m – arithmetic mean error, CV – coefficient of variation. Anatomic parameters are considered low-variance at CV < 20%, medium-variance – at CV = 20–40%, and high-variance – at CV > 40 % (Butnik and Timchenko 1987). Data correspond to normal distribution. To assess the significance of variance in independent samples, the authors calculated a t-test statistic value assuming equal variance in samples; a t-test statistic value assuming unequal variance in samples; an F-test statistic value. The significant variance was determined at the confidence level of P < 0.05.

Results

Waldsteinia ternata grown under different light conditions were different in the external dimensions of leaves, as well as in the size and shape of their anatomical structures.

The cells of the upper and lower epidermis of plants grown in the sun exhibit a less sinuous shape (Fig. 1) and small size (Table 1). The number of cells of the upper epidermis of plants grown in the sun is 565.76 ± 12.93 cells/mm^2, and the cell size attains 1917.38 ± 127.29 mm^2. The cells of plants grown in the shade are significantly larger in size (2386.31 ± 124.52 mm^2) and, therefore, their number per unit area is less and equals 420.48 ± 8.84 cells/mm^2. Moreover, the upper epidermis of plants grown in the sun is thicker (26.84 ± 0.95 μm) than that in plants grown in the shade (23.20 ± 0.66 μm). A similar pattern can be observed for the size and number of cells of the lower epidermis. The size of cells of both the upper and lower epidermis is characterized by a medium and high variation level, especially for cells of the lower epidermis. The thickness of the upper and lower epidermis, as well as the number of epidermal cells, is more stable (coefficient of variation does not exceed 20%).

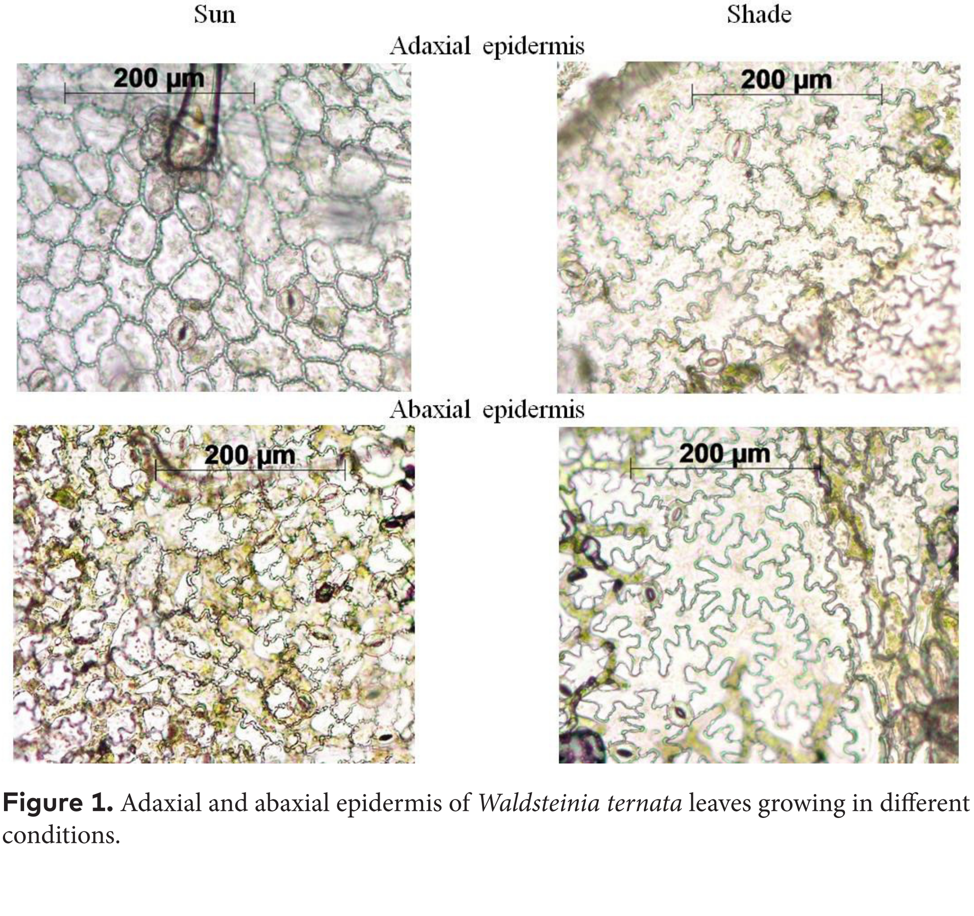

Figure 1. Adaxial and abaxial epidermis of *Waldsteinia ternata* leaves growing in different conditions.

Table 1. *Waldsteinia ternata* leaf epidermis anatomy characteristics growing in different conditions

Characteristic	Sun	Shade
	M ± m CV, %	
Number of adaxial epidermal cells per 1 mm^2, pcs	$565.76^{A} \pm 12.93$ 11.4	$420.48^{B} \pm 8.84$ 10.5
Number of adaxial epidermis stomata per 1 mm^2, pcs	$51.20^{A} \pm 5.99$ 58.5	$19.84^{B} \pm 3.37$ 84.8
Adaxial epidermis stomatal index, %	$7.96^{A} \pm 0.85$ 53.1	$4.33^{B} \pm 0.69$ 79.4
Adaxial epidermal cell size (area), μm^2	$1917.38^{A} \pm 127.29$ 33.2	$2386.31^{B} \pm 124.52$ 26.1
Adaxial epidermis stomata length, µm	$32.76^{A} \pm 0.40$ 6.0	$30.33^{B} \pm 0.33$ 5.4
Adaxial epidermis stomata width, µm	$28.50^{A} \pm 0.38$ 6.7	$26.08^{B} \pm 0.40$ 7.6
Adaxial epidermis thickness, µm	$26.84^{A} \pm 0.95$ 17.7	$23.20^{B} \pm 0.66$ 14.3
Number of adaxial epidermal trichomes per 1 mm^2, pcs	$6.72^{A} \pm 1.58$ 117.4	$10.24^{A} \pm 2.55$ 124.6
Number of abaxial epidermal cells per 1 mm^2, pcs	$649.60^{A} \pm 22.72$ 13.5	$359.04^{B} \pm 14.07$ 19.6
Number of abaxial epidermis stomata per 1 mm^2, pcs	$121.60^{A} \pm 6.77$ 21.6	$67.52^{B} \pm 6.40$ 47.4
Abaxial epidermis stomatal index, %	$15.86^{A} \pm 0.91$ 22.3	$15.17^{A} \pm 1.17$ 38.5
Abaxial epidermal cell size (area), μm^2	$1739.15^{A} \pm 390.25$ 92.2	$3360.88^{B} \pm 281.65$ 41.9
Abaxial epidermis stomata length, µm	$30.93^{A} \pm 0.39$ 6.2	$30.09^{A} \pm 0.38$ 6.4
Abaxial epidermis stomata width, µm	$25.47^{A} \pm 0.31$ 6.2	$23.55^{B} \pm 0.41$ 8.7
Abaxial epidermis thickness, µm	$25.30^{A} \pm 0.84$ 16.6	$16.08^{B} \pm 0.38$ 11.9
Number of abaxial epidermal trichomes per 1 mm^2, pcs	$3.20^{A} \pm 1.71$ 207.0	$2.24^{A} \pm 1.09$ 242.2

Note: Different letters show significant variance at the confidence level of $P < 0.05$.

The leaves of *W. ternata* are amphistomatous and are characterized by anomocytic type stomata. A greater number of stomata of larger sizes (51.20 ± 5.99 pcs/mm^2, 32.76 ± 0.40 µm in length and 28.50 ± 0.38 µm in width) were observed on the upper epidermis of plants grown in the sun compared to those grown in the shade (19.84 ± 3.37 pcs/mm^2, 30.33 ± 0.33 µm in length and 26.08 ± 0.40 µm in width).

Consequently, the stomatal index of the upper epidermis of plants grown in the sun is higher (7.96 ± 0.85%) than that of plants grown in the shade (4.33 ± 0.69%). The stomata are unevenly distributed; therefore, the data obtained are not uniform. A similar pattern can be observed for the lower epidermis; however, the stomatal index of plants grown in the sun and in the shade does not differ significantly (15.86 ± 0.91% and 15.17 ± 1.17%, respectively), as well as the stomata length (Table 1). A varying number of single trichomes, with distribution of values different from normal, are found on the upper and lower epidermis.

The dorsoventral leaves of *W. ternata* (Fig. 2) are thinner in the shade (Table 2), the thickness of the central vein is 339.99 ± 13.67 μm, and the thickness of the blade is 166.82 ± 1.92 μm, versus 400.69 ± 7.15 μm and 283.67 ± 5.22 μm, respectively, for plants grown in the sun. The thickness of the mesophyll (including the palisade and spongy mesophyll) is 2 fold greater than that in plants grown in the sun. At the same time, the ratio of palisade to spongy mesophyll does not reveal any significant differences and attains 0.70 ± 0.03 for plants grown in the sun and 0.67 ± 0.03 for plants grown in the shade. The size of cells of the upper layer of the mesophyll, the most photosynthetically active tissue, is larger in plants grown in the sun (the length of cells in leaves of plants grown under good light conditions is 66.88 ± 1.99 μm versus 42.12 ± 0.98 μm in leaves of plants grown in the shade).

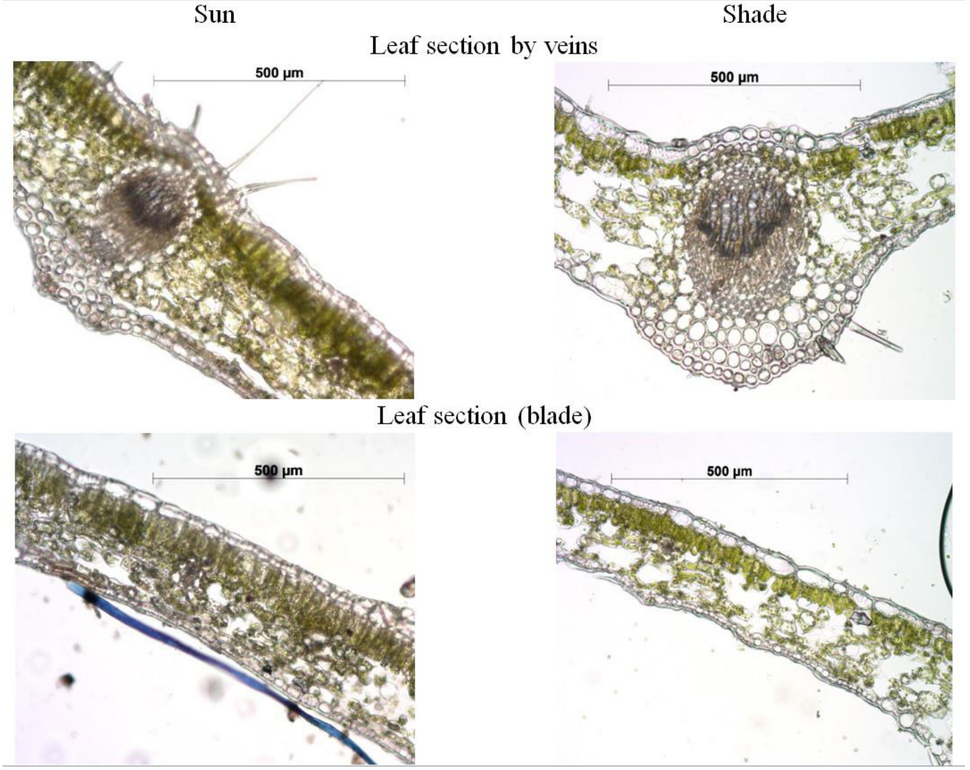

Figure 2. *Waldsteinia ternata* leaf blade anatomy growing in different conditions.

Table 2. *Waldsteinia ternata* leaf anatomy characteristics

Characteristic	Sun	Shade
	M ± m CV, %	
Leaf thickness (midrib), μm	400.69^{A} ± 7.15 8.9	339.99^{B} ± 13.67 18.0
Leaf thickness (blade), μm	283.67^{A} ± 5.22 9.2	166.82^{B} ± 1.92 5.8
Mesophyll thickness, μm	235.46^{A} ± 4.94 10.5	128.44^{B} ± 1.68 6.6
Palisade mesophyll thickness, μm	94.28^{A} ± 2.75 14.6	50.29^{B} ± 1.15 11.5
Spongy mesophyll thickness, μm	142.27^{A} ± 4.40 15.5	76.61^{B} ± 1.69 11.0
Palisade mesophyll / spongy mesophyll ratio	0.70^{A} ± 0.03 24.6	0.67^{A} ± 0.03 19.8
Cell length of the upper mesophyll layer, μm	66.88^{A} ± 1.99 14.8	42.12^{B} ± 0.98 11.6
Cell width of the upper mesophyll layer, μm	22.47^{A} ± 0.73 16.1	19.20^{B} ± 0.62 16.1
Bundle cross section area, $μm^2$	18415.44^{A} ± 852.37 23.1	26349.69^{A} ± 6066.76 72.8
Xylem cross section area, $μm^2$	8496.64^{A} ± 376.86 22.2	13788.57^{B} ± 3309.62 75.9
Phloem cross section area, $μm^2$	7613.60^{A} ± 421.72 27.7	11133.07^{B} ± 2526.285 71.8
Xylem/phloem area ratio	1.15^{A} ± 0.04 17.2	1.21^{A} ± 0.05 14.0

Note: Different letters show significant variance at the confidence level of $P < 0.05$.

Despite a significant difference in the dimensions of the central vascular bundle (18415.44 ± 852.37 $μm^2$ in plants grown in the sun, and 26349.69 ± 6066.76 $μm^2$ in plants grown in the shade), these dimensions do not show significant differences in plants grown under different conditions. This is due to strong variation in the size of the vascular bundle in leaves of plants grown in the shade (CV = 75.9%).

The leaves of plants grown in the shade are distinguished by the large size of the xylem and phloem, which attains 13788.57 ± 3309.62 $μm^2$ and 11133.07 ± 2526.285 $μm^2$, respectively, versus 8496.64 ± 376.86 $μm^2$ and 7613.60 ± 421.72 $μm^2$ for plants grown in the sun. The sizes of the vascular tissues of plants grown in the shade, as well as the vascular bundle, exhibit a high coefficient of variation exceeding 70%. In this case, the ratio of vascular tissues is similar for plants grown under different conditions (Table 2).

Discussion

Based on the study, the plants can be referred to mesophytes (dorsoventral leaves, moderate development of integumentary, vascular, palisade and spongy tissues, presence of stomata on the lower epidermis, etc.) (Prokopiev 2001).

Waldsteinia ternata grown in the sun exhibited an increased number of stomata and epidermal cells, an increased thickness of the leaf and mesophyll, and an increased number of cells of the upper and lower epidermis. This indicates heliophytic features of plant adaptation to good light conditions, which is consistent with similar studies carried out for other plant species (Boardman 1977; Givnish 1988; Aleric and Kirkman 2005; Li et al. 2018). At the same time, plants grown in the sun had less developed vascular tissues, which indicates their adaptation to unfavorable water conditions and ensures the maintenance of a normal water balance, since good light conditions often cause water deficit (Kleinwächter and Selmar 2015).

A number of relative indicators, such as the stomatal index of the lower epidermis, the ratio of palisade to spongy mesophyll, and the ratio of xylem to phloem, were not affected by different growth conditions.

Conclusion

The relationship between the development of the anatomical structure of leaves and light conditions has been established.

W. ternata acquires helioxeromorphic or sciomesomorphic features when exposed to different light and water conditions. Shaded conditions are most favorable for plant introduction in the forest zone of Western Siberia, which corresponds to the phytocenotic confinement of the species.

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