CZECH POLAR REPORTS 13 (1): 24-36, 2023

Comparative anatomical and physiological characteristics of *Ranunculus glacialis* and estimation of its adaptive potential in natural habitats and the PABGI nursery (Murmansk region)

Natalya Yu. Shmakova[*], Olga V. Ermolaeva

N. A. Avrorin Polar-Alpine Botanical Garden-Institute, Kola Science Centre Russian, Academy of Sciences, Fersman street 18a, Apatity, Murmansk region, 184209, Russia

Abstract

Ranunculus glacialis (L.) A. Löve & D. Löve is a rare species that is included in the Red Data Book of the Murmansk region. It belongs to a group of northern species that, under climate change conditions, will be exposed to a reduction of range and loss of genetic diversity. The objective of this study was to estimate the adaptive potential of this species in the Khibiny Mountains, which is the edge of the eastern limit of its range. Plants growing in natural conditions of the Khibiny Mountains and in the nurseries of the Polar-Alpine Botanical Garden-Institute (PABGI) were compared in terms of leaf mesostructure and pigment content. Under nursery conditions, at higher temperature than in the field, *R. glacialis* plants showed quantitative rearrangement of leaf mesostructure. Changes associated with increases in internal leaf volume and disturbance of ontogeny, changes in morphometric indicators of assimilating organs (mass and leaf area), reduced productivity and, consequently, reduced resistance to growing conditions were also found in the PABGI-cultivated plants. In this study, we show that this species has a low level of genetic diversity and a limited adaptive potential in the extreme eastern edge of its range in Russia (Kola Peninsula), as evidenced by numerous experiments on acclimatization of *R. glacialis* under nursery conditions in the Khibiny Mountains.

Key words: *Ranunculus glacialis*, leaf anatomy, biometric parameters, chlorophylls, carotenoids, nursery, natural site, Murmansk region

DOI: 10.5817/CPR2023-1-3

Introduction

Climate change will lead to range shrinkage of multiple species and a loss of the genetic diversity that determines their long-term survival. Genetic diversity is essential not only for the sustainability of a species and its evolutionary potential but also for community structure and ecosystem stability (Hughes et al. 2008). Most species adapted to cold environments are expected to suffer range reduction fol-

---

Received February 6, 2023, *accepted* May 19, 2023.

[*] Corresponding author: N. Shmakova <shmanatalya@yandex.ru>

Acknowledgements: The work was supported by the RSF grant (Russian Science Foundation) No. 22-14-20002 (“Biological diversity and functioning of the Arctic mountain ecosystems of the Kola Peninsula in the era of global climate change”). We thank E. Markovskaya for constructive and useful comments of this paper. The authors would like to thank Falcon Scientific Editing for proofreading the English language in this paper.

lowing climate warming (Parmesan et al. 2006). The study of Alsos et al. (2012) focused on 27 plant species from northern territories that are most vulnerable to present-day climate change. The modeling used by the authors showed that all species will experience range shrinkage and genetic diversity losses. Grass plants, which are not adapted to long-distance dispersal, will lose genetic diversity at a greater rate than shrubs. However, the authors note the high prediction uncertainty for two species, *Arabis alpina* and *Ranunculus glacialis*; the latter is of interest to us. The peculiarity of these species is their high level of genetic diversity in the southern alpine areas and almost no diversity in the northern areas. This characteristic defines their uniqueness (Alsos et al. 2012). Because genetic fragments of southern populations are frequently lost, the expected loss of genetic diversity in these species may cause serious genetic depletion of the species. This prediction is based on loss of genetic diversity, whereas the ability to adapt and survive climate change mainly depends on adaptive genetic variability (Bonin et al. 2007). The ability to assess this adaptive component for *Ranunculus glacialis* under subarctic conditions is of considerable interest.

Arctic plants are characterized by polyvariance of development, which includes the development of shoot systems, changes in the age structure of populations, reduction of life cycle, increase in the duration of development, and transition from seed to vegetative reproduction. Polyvariance is dependent on environmental factors, temperature in particular. The study of the age structure of *R. glacialis* polulation in the Lovozero tundra region (Kola Peninsula) shows a reduction in the life cycle, the predominance of individuals of the pregenerative period (68-88%), which is typical for invasive, developing populations. Active seed reproduction under the conditions of the mountain Arctic desert indicates a high tolerance and ecological plasticity of this species. There are no individuals in late stages of ontogenesis in the cenopopulations (Vasilevskaya 2007).

Physiological studies an *R. glacialis* from different altitudes of the Alps showed its resistance to high light intensities at low temperature, the ability to keep photosynthetic electron transport chain active, and dominance of malate as the main source of carbon and transport metabolite (Streb et al. 1998). In the European Alps, only a few plant species exist at 4000 m a.s.l., for example, *Ranunculus glacialis*. Previous studies have shown that *R. glacialis* has an extremely conservative growth strategy and low developmental plasticity in response to different periods of snowmelt (Wagner et al. 2010). Under high-altitude conditions, the species shows an increase in leaf size and thickness compared to plants transferred to low latitudes with higher temperatures. However, the high resistance of *R. glacialis* leaves to high light exposure is not related to an increase in antioxidant protection (Streb et al. 1998). Low thermal dissipation values, high electron transport rate (ETR), and low photorespiration values have been noted for this species, which distinguishes it from the others. Non-photochemical fluorescence quenching is of minor importance for *R. glacialis* photoprotection (Streb et al. 1998). One reason that may explain the high functional activity of the photosynthetic apparatus of this species is the high content of plastid terminal oxidase (PTOX). It exceeds the values obtained in other species and is even higher than that in the leaves of transgenic tomatoes with PTOX protein overexpression (Streb et al. 2005). Thus, the PTOX content considerably decreases during deacclimatization of *R. glacialis*. Associated with this is the increased susceptibility of *R. glacialis* leaves to photoinhibition induced by light at higher temperatures and the inability to repair damage caused by high temperatures, which may limit the spread of *R. glacialis* at lower altitudes in the Alps (Streb et al. 2003).

The experimental conditions at PABGI (Khibiny Mountains) are unique because they allow comparative studies at high altitudes and at experimental sites with small altitude variation. Cultivation of plants at the nurseries of the Polar-Alpine Botanical Garden-Institute (PABGI) is considered one of the ways to conserve this species. The aim of this study was to estimate the adaptive potential of the species when comparing the features of ecological and physiological parameters of *Ranunculus glacialis* under high-altitude conditions and in the PABGI nursery in the Khibiny Mountains (Aikuaivenchorr Mountain) in the Murmansk region (67°38'48"N, 33°38' 51"E).

Material and Methods

Description of the species. *Ranunculus glacialis* L. (=*Beckwithia glacialis* (L.) Á. et D. Löve) is an arctic-alpine amphiatlantic species of the Ranunculaceae family. *R. glacialis* is a 5–15 cm-tall herbaceous perennial with a shortened rhizome, numerous adventitious roots, and a rosette of carnose root leaves with a 1–2-cm-long and 2–3cm-wide dissected blade and a long petiole that is broadened into a membranous sheath at the base. It has large white or pink flowers that are up to 2.5 cm in diameter, solitary (sometimes 2–3 flowers). The populations are formed by differently-aged individuals. They are characterized by slow development at early stages of ontogenesis; the considerable mortality of seedlings and juvenile individuals is compensated by relatively good seed productivity (Red Data Book 2014[1]). The vegetation period usually begins 5–10 days after melting of snow (in places with late snowmelt, regrowth begins already under the snow) and lasts 2–2.5 months. Spring vegetation of the species, *i.e.* with the expansion of the leaves marking the beginning of the plant's budding stage, which is not pronounced, lasts 8–27 days. Flowering begins in late June to early July. The maturation fruit period lasts 2–3 weeks, and the leaves begin to change color in late July/early August, with dark spots appearing on them as a result of the frost-induced effects on leaf tissue common at this time. The plants usually become brown in the first half of August and enter the phase of autumn dormancy in mid-August (Andreeva 1985).

Distribution. *Ranunculus glacialis* is one of the Alpine plant species in the central European Alps. It grows at the highest altitude (2370 m a.s.l.) of any vascular plants in Scandinavia. It has the lowest July temperature optimum (7.3°C) of the Norwegian alpine flora (Totland and Alatalo 2002). *R. glacialis* grows on coarse moraine, rocky placers with patches of fine earth between rock, rocky ledgesas well as on freshly soaked and moist, well drained, acidic silicate debris. All habitats are characterized by humus-poor soils and thinned vegetation cover (Red Data Book 2014[1], Koroleva and Kopeina 2020). In Russia, *R. glacialis* occurs only in the Murmansk region in the Khibiny and the Lovosersky Mountains in the belt of goltsy deserts and in the mountain tundra. In such ecosystems, *R. glacialis* grows on coarse grained debris on steep slopes, in places of late snowmelt. Earlier study of Koroleva and Kopeina (2020) ascribed the community type not to snow bed vegetation but to *Racomitrium* spp. – *Ranunculus glacialis* of order *Androsacetalia alpinae* and class *Thlaspietearotundifolii*. *R. glacialis* is a species of the Red Data Book of Murmansk region (RDBMu): 2 (VU – Vulnerable, including declining in numbers).

Study site description. The habitats of natural growth of *R. glacialis* (the south-west slope of the Aikuaivenchorr Mountain at an altitude of 750 m a.s.l., Fig. 1 B) are characterized by the following temperature conditions: average annual – (-4.9°C), average for the growing season – 4.0°C, and the warmest month is July – 7.5°C. The annual amount of precipitation is 1461 mm, during the growing season – 526 mm (Table 1). Relative air humidity (RH) during the growing season varies between 84% and 90%. The increase in relative humidity is associated with the rise of warm air along the mountain slopes due to its cooling in the process of ascent. The snow-free period varies from 70 to 100 days. The main meteorological factors in this area are given according to data from the mountain avalanche station 'Tsentralnaya', because the height and exposition of the slopes are similar to those of the Aikuaivenchorr Mountain (Zyuzin 2006).

The PABGI nursery (Fig. 1 A) is located at the foot of the Vudjavrchorr Mountain (Khibiny) in the forest belt at an altitude of 320 m a.s.l. The area is characterized by the following temperature conditions: average annual – (-0.5°C), average for the growing season – 9.9°C, and the warmest month is July – 15.3°C. The annual amount of precipitation is 900 mm, during the growing season – 282 mm (Table 1). RH during the growing season varies between 64% and 85%. The snow-free period varies from 97 to 122 days, and the frost-free period lasts 2.5–3 months; however, ground frost is possible on any day of summer. There may be occasional droughts of up to 2–3 weeks (Semko 1989).

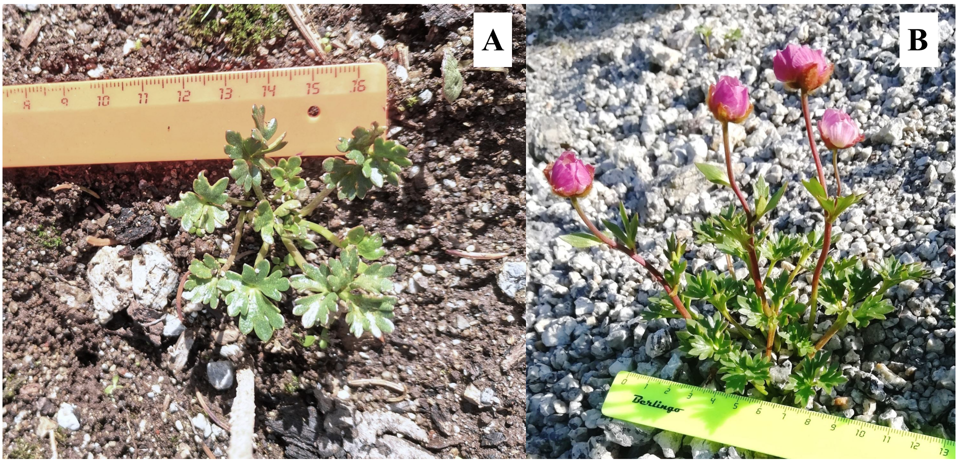

Fig. 1. *Ranunculus glacialis* (L.) Á. Löve & D. Löve cultivated in PABGI nursery (A) and in natural habitat (B).

Methods. Anatomical parameters (for the list see below) were studied in the 4th year of cultivation of *R. glacialis* individuals in the PABGI nurseries on leaves that had completed their growth according to Mokronosov and Borzenkova (1978). The leaf blade was divided into three deeply dissected dentate segments. Sections from the middle part of dentate segments (n=10 leaves) were analyzed using a MIKMED-6 light microscope (LOMO, Russia). The parameters (leaf thickness, stomata size, and tissue cell size) were measured using a WF10X/22mm ocular micrometer. Longitudinal paradermal sections were used to determine the type of stomatal apparatus, number and size of stomates (Sautkina and Polyksenova 2011).

The content of photosynthetic pigments of leaves was determined in ethanol extracts (96%) by the measurements of the absorption maxima of chlorophylls *a* and *b* and carotenoids (Lichtenthaler and Wellburn 1983, Maslova and Popova 1993) using a UV-1800 spectrophotometer (Shimadzu, Japan). Leaves of each species were sampled in 5-fold biological replicates and then analyzed using 3-fold analytical replicates.

The leaf area (mean of at least 10 fully developed leaves) was determined by computer scanning in the ImageJ program (LOCI, University of Wisconsin, USA). Dry matter of leaf area unit was determined by drying at 105°C to absolute dry weight (DW). Specific leaf weight (SLW, g dm^{-2}) was calculated as the ratio of leaf DW per area.

Statistical data processing was performed using the standard software package in Microsoft Excel 7. Level of a variation of parameters was estimated using coefficient of variance which is calculated as CV=σ/M×100%, where σ is a standard deviation, M is an arithmetic average (Ivanter and Korosov 2010). Student's t-test was used to assess the statistical significance of the parameters.

Indices	June		July		August		September	
	1	2	1	2	1	2	1	2
Monthly mean air temperature, °C	3.6	11.7	7.5	15.3	5.0	12.0	–0.1	6.7
Precipitation, mm	116	71.3	89.4	68.7	129.8	79.0	191	62.9
Snow-free period, days	70-100	97-122						

Table 1. Characteristics of the habitat conditions of *R. glacialis*. *Note*: 1 – Natural habitat (Zyuzin 2006), 2 – In the nursery (PABGI, own data).

Results

Mesostructure of the *R. glacialis* leaves in the PABGI nursery. The mature leaf of a vegetative individual has a typical dorsoventral structure (Fig. 2). The thickness of the leaf blade averaged 862±15 µm (Table 2), with a low coefficient of variation (CV = 6%). The assimilatory tissue of the leaf blade was clearly differentiated into a palisade and spongy mesophyll. The palisade mesophyll was developed on the adaxial side of the leaf, while the spongy mesophyll on the abaxial side. Leaf palisade mesophyll consisted of three rows of densely arranged elongated cells (the average shape index is 3.8). The average layer thickness was 389±9 µm; the layer was formed by cells with an average volume of 40 thousand µm^3. Conducting bundles appeared at the interface between the palisade and the spongy layer of mesophyll cells. The average spongy layer thickness was 440±25 µm, and it was formed by cells whose volume varied to a large extent (CV = 65.2%). Intercellular gaps were formed between the cells of the spongy mesophyll. The palisade coefficient in this species (*i.e.* the ratio of palisade thickness to spongy tissue thickness) was quite high (0.88). The number of chloroplasts in cells varied from 55 to 80. Although their number in the palisade layer was 1.2 times higher than in the spongy layer, the cell volume corresponding to one chloroplast (chloroplast cell volume, CCV) was similar in all mesophyll cells. The obtained coefficient of variation of CCV indicated the relative heterogeneity of this index (CV – on average 33%).

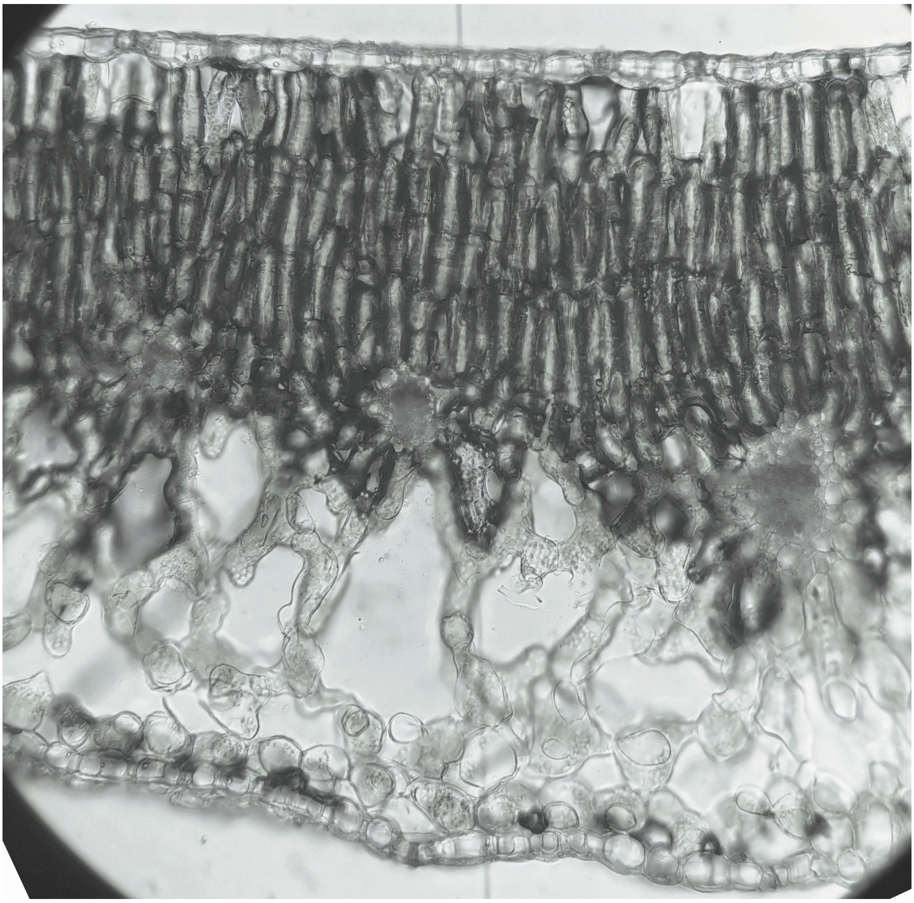

Fig. 2. Fragment of leaf cross-section of *R. glacialis* (photo taken at 10× magnification).

The cover tissue on a cross section was represented by a single-layer upper and lower epidermis formed by round-quadratic or rectangular cells. The upper epidermal cells (25.7±0.3 μm) were thicker than the lower epidermal cells (22.0±0.4 μm); the shape indices of the cells (length-to-width ratio) reached 1.2 and 1.3, respectively. Epidermal cell length had more variable index (CV – up to 27%), than cell width (CV – up to 14%). Both sides of the epidermis had irregularly arranged stomata. The stomata were surrounded by an indeterminate number of cells, which did not differ in shape or size from the main cells of the cover tissue (anomocyte type of stomatal apparatus). The cells on the lower epidermis were more sinuous than those on the upper epidermis (Fig. 3 A and B). Guard cells did possess chloroplasts. The stomata on the upper epidermis were smaller area (1311±24 μm^2) than those on the lower epidermis (1501±30 μm^2). Their number was 2.4 times greater than that in the lower epidermis (139±3 and 58±3 units mm^{-2}, respectively). A high coefficient of variation in the number of stomata observed on the lower epidermis (CV = 21.9%) indicated their irregular distribution. The shape index of the stomata was 1.2.

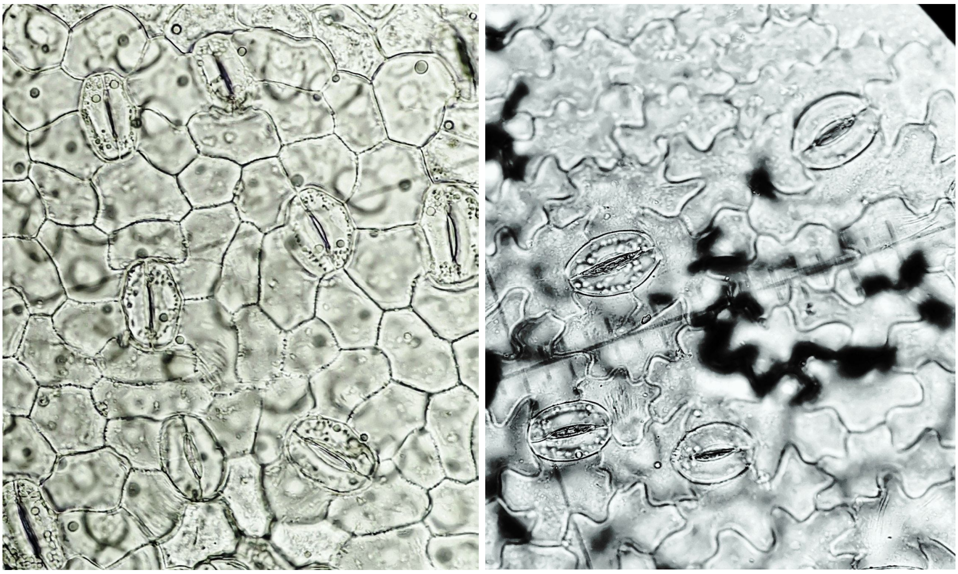

Fig. 3. Fragment of the leaf epidermis of *R. glacialis*: A – upper, B – lower (photo taken at 40× magnification).

Mesostructure of the *R. glacialis* from the natural habitat. The leaf has a dorsoventral structure. The average thickness of the leaf blade was 760±30 μm (Table 2), with a low coefficient of variation (CV = 5.5%). The palisade leaf mesophyll clearly showed three rows of densely arranged elongated cells (average shape index of 4.4). The average layer thickness was 351±4 μm; the layer was formed by cells with an average volume of 17 thousand μm^3. The average thickness of the spongy layer was 358±11 μm. The indicator of cell volume had a high proportion of scatter from the average value, indicating the relative heterogeneity of this trait (CV – on average 51%). The palisade coefficient in this species in the natural habitat was 0.98.

The number of chloroplasts in cells varied from 44 to 63. The palisade layer had 1.3 times more chloroplasts than the spongy layer. The average CCV value was 293 μm^3 and thus slightly greater in the spongy layer than in the palisade layer.

The cover tissue was represented by single-layered upper and lower epidermis with round-quadratic cells (shape indices are 0.9 and 1.0, respectively). The cells of the upper epidermis were 1.1 times thicker than those of the lower epidermis (25.8± 0.3 and 22.8±0.5 μm, respectively). The width of epidermal cells had a low coefficient of variation (CV – up to 10%), indicating the relative homogeneity of this trait. The stomata were irregularly arranged on both sides of the epidermis. The cells on the lower epidermis were more sinuous than those on the upper epidermis. The area of stomata on the upper epidermis (920±16 μm^2) was smaller than those on the lower epidermis (1230±24 μm^2). The shape of the stomata was 1.4. Their number was 2.9 times greater than in the lower epidermis (228±5 and 78±7 units mm^{-2}, respectively). The coefficient of variation in the number of stomata does not exceed 20%.

Indices	Cultivated in the nursery (PABGI, Khibiny)		Natural habitat (Aikuaivenchorr Mt, Khibiny)	
	M±m	CV, %	M±m	CV, %
Leaf area, cm^2	2.66±0.19	42.1	3.15±0.18	30.5
Thickness, µm:				
leaf	862±15***	6	760±13	5.5
upper epidermis (U)	25.7±0.3	12	25.8±0.3	7.5
lower epidermis (L)	22.0±0.4	14.2	22.8±0.5	9.8
Number of stomata, pieces mm^{-2},U/L	139±3*** 58±3***	10.8 21.9	228±5 78±2	16.9 19.3
Stomata size, µm:				
length/width, U	43.7±0.4*** 38.0±0.4***	8.6 8.6	40.0±0.4 29.2±0.4	10.6 14
length/width, L	47.1±0.5 40.5±0.6***	7.3 9.3	46.6±0.4 33.5±0.5	9.1 14.4
Stomata area, µm^2, U/L	1311±24*** 1501±30***	15.4 13.6	920±16 1230±24	20.3 20.2
Palisade mesophyll (P):				
thickness, µm	388.8±8.8***	6.3	350.9±3.9	3.7
number of layers, pieces	3	–	3	–
cell volume, thousand µm^3	39.2±1.3***	38.3	17.2±1.6	51.8
Spongy mesophyll (S):				
thickness, µm	440.0±25.4**	16.3	358.2±11.3	10.4
cell volume, thousand µm^3	29.5±2.5***	65.2	15.3±1.6	49.7
Cell number, thousand cm^{-2}, P/S	77.3±3.3** 57.0±2.4*	7.4 7.3	118.8±7.8 69.5±4.1	16.1 14.2
Chloroplast number per cell, pieces, P/S	76±4** 62±7*	17.4 23.7	61±2 47±3	18.5 29.1
Chloroplast number, million cm^{-2}	9.4	–	10.5	–
Cell volume corresponding to 1 chloroplast, µm^3, P/S	465±50*** 475±64*	34.4 32.8	272±18 314±20	36.3 28.6

Table 2. Indices of leaf mesostructure of *Ranunculus glacialis*. *Note*: M±m – mean±standard error; CV – coefficient of variation. Differences between habitats are significant: * – at $p \leq 0.05$, ** – at $p \leq 0.01$, *** – at $p \leq 0.001$.

Photosynthetic pigment content of *R. glacialis* leaves

The chlorophyll content of leaves *R. glacialis* grown in the PABGI nursery during the years of research ranged from 3.0 to 6.5 mg g^{-1} DW, and the content of carotenoids was 0.7–1.4 mg g^{-1} DW. During the vegetation phase, photosynthetic pigment content reached chlorophyll contents of 4.86 chlorophylls mg g^{-1} and carotenoids contets of 1.01 mg g^{-1} DW (Table 3). In the flowering phase of *R. glacialis* under culture conditions (June 26th–July 5th), the chlorophyll content was 4.5–5.0 mg g^{-1} DW, and the carotenoid content was 1.02–1.08 mg g^{-1} DW. The highest pigment content was recorded for late June–early July. A noticeable decrease in green and yellow pigments of plastids was found already in mid–July. By the end of July, the chlorophyll and carotenoids content was 50% and 40% lower compared with the maximum values. The fruiting phase may occur during this period. The minimum chlorophyll content of approximately 3 mg g^{-1} DW was typical for late August. The chlorophylls (*a/b*) ratio is 3.3 and the chlorophylls/carotenoids ratio is 4.8. Dry matter content of *R. glacialis* leaves was stable and is an average of 18.7%. The average DW of a leaf reached 20.4 mg, and SLW reached 0.77 g dm^{-2} (Table 3).

In natural conditions on rocky placers with patches of fine earth in goldsy deserts (Khibiny, Aikuaivenchorr Mt., 750 m a.s.l.), *R. glacialis* had contents of chlorophylls and carotenoids 3.95 and 1.05 mg g^{-1} DW, respectively, during vegetation. During flowering, it reached 4.87 and 1.4 mg g^{-1} DW. The chlorophylls (*a/b*) ratio is 3.3, and the chlorophyll/carotenoids ratio was 3.8. The dry matter content of *Ranunculus glacialis* leaves reached 17–20% of total DW. The *R. glacialis* leaf weight was 22.6 mg, and the SLW reached 0.72 g dm^{-2} (Table 3).

Indices	Cultivated in the nursery (PABGI, Khibiny)	Natural habitat (Aikuaivenchorr Mt, Khibiny)
Leaf weight, mg dry weight	20.4±7.9	22.6±2.9
Specific leaf density, LMA, (dry weight/area), g dm^{-2}	0.77±0.10	0.72±0.08
Chlorophylls (*a*+*b*), mg g^{-1} dry weight	4.86±1.02*	3.95±0.75
Chlorophylls (*a*+*b*), mg dm^{-2}	3.74±0.67*	2.84±0.46
Chlorophylls content of in 1 chloroplast, 10^{-9} mg	3.98*	2.70
Carotenoids, mg g^{-1} dry weight	1.01±0.16	1.05±0.08
Chlorophylls *a* and *b* ratio / chlorophylls and carotenoids ratio	3.3/4.8	3.3/3.8

Table 3. Morphometric parameters and photosynthetic pigments content of *Ranunculus glacialis* leaves in different habitats. *Note*: Data – mean ± standard deviation. Differences between habitats are significant: * – at $p \leq 0.05$. The content of photosynthetic pigments was determined during the vegetation phase.

Discussion

Ranunculus glacialis belongs to the group of high altitude plants adapted to a complex of peculiar environmental conditions: *i.e.* long winter, short vegetation period, low soil and air temperatures, high insolation, and wetting due to spring snowmelt. In their natural habitat, *R. glacialis* plants have a thick leaf blade and multilayered palisade tissue consisting of small cells, which is indicative of adaptation of the plants to the arid and cold conditions of highlands (Kudryavtseva et al. 2001). A high palisadity coefficient, high density of spongy tissue cells, and a large number of small stomata are adaptations to environmental conditions in order to limit transpiration and aeration of internal tissues (Ramazanova and Asadulaev 2012). The lustrous leaf surface is an additional structure for weakening light penetration to chloroplasts.

In the plants cultivated in a nursery, the leaves of *R. glacialis* were smaller but thicker. Streb et al. (2003) noted a decrease in the leaf size of *Ranunculus glacialis* and the loss of their succulence in deacclimatization experiments at 22°C. The leaf adaptation to high insolation and water deficit is accompanied by an increase in the cell density (number of cells per unit leaf area)(Mokronosov 1978, Ivanov et al. 2004, Ivanova 2014). There was a significant increase in the volume (2.1 times on average) of mesophyll cells with a decrease in their number per unit area (1.5 times in the palisade layer and 1.2 times in the spongy layer). The analysis of spongy layer parameters (thickness, volume, and number of cells) indicated that plants from the nursery have a more developed system of intercellular ducts representing air cells. Stomata had a markedly larger size and area, while their number is lower (1.6 times), especially on the upper epidermis. The increase in stomata area indicated the large gas exchange capacity of single stomata. The number of chloroplasts in cells was slightly higher (1.2 times) compared to that in the natural habitat. An average of 1.6 times increase in the CCV index indicated a smaller size of green plastids in nursery plants. Meanwhile, the chloroplast had to support a larger cell volume, which ultimately leads to inhibition of growth processes (Nakonechnaya et al. 2019).

Comparison of morphometric indices revealed a decrease in leaf DW and leaf area under culture conditions in the PABGI nursery. These indices are believed to have a positive relationship, but SLW is more frequently associated with leaf thickness (Vasfilov 2011). A 2- to 2.2-fold increase in cell volume in the palisade and spongy parenchyma in the PABGI plants compared to those from natural habitat resulted in increased leaf thickness and SLW. This occured possibly owing to a change in precipitation regime (under nursery conditions, the amount of precipitation was 2 times lower (Table 1) than that under Aikuaivenchorr Mountain conditions). In heliophytic species, shading can lead to a decrease in leaf area, which was found in *R. glacialis* grown under nursery conditions. Reduced leaf area leads to increased heat dissipation and reduced transpiration, which is important under droughty conditions (Ivanova 2014).

These studies show that the Red Data Book species *R. glacialis* (rank among rare spacies inthere) belongs to the group of stenobionts species with specific physiological features under conditions of the Khibiny Mountains (which is the eastern edge of its range). It allows the species to adapt to extremely harsh environmental and poor highland soil conditions. In terms of the functional activity of the photosynthetic apparatus, its adaptations include a wide range of defense mechanisms. Therefore it likely belongs to the group of stenobionts species. In the natural habitat, the chlorophyll content of *R. glacialis* leaves was 1.2–1.3 times lower than under culture conditions. The change in the content of chlorophylls is associated with a decrease in their amount in one chloroplast by 1.5 times (Table 3). No significant differences were found in the content of carotenoids in leaves from different habitats. However, the diffence found in the chlorophyll/carotenoids ratio supports a large participation of yellow pigments in the protection of the photosynthetic apparatus from high illumination on the Aikuaivenchorr Mountain (more than 1000 μmol m^{-2} s^{-1}). Changes in the ratio of pigment types are associated with a restructuring of the light-harvesting complex within chloroplasts (Ivanov et al. 2013), with changes in chlorophyll content per unit leaf area being typically due to quantitative changes in cells and tissues (Mokronosov 1981).

A change in the chlorophyll content with an increase in airtemperature of the habitat was experienced in the experiment involving the transfer of *Ranunculus glacialis* specimens from the French Alps (2400–2700 m a.s.l.) to other conditions of existence (200 m a.s.l., 22°C and 6°C). Individuals showed no changes in morphological or biochemical parameters at low temperature (6°C), whereas at 22°C, the main parameters differed significantly from natural. The chlorophyll content of *R. glacialis* leaves under natural conditions of high altitude was 0.9 mg g^{-1} of fresh weight, whereas it was 0.8 mg g^{-1} of fresh weight at 6°C and 1.5 mg g^{-1} of fresh weight at 22°C (Streb et al. 2003). The authors showed that deacclimatization of the species depended to a greater extent on temperature, and not on the height of growth.

In 2021, measurements of net photosynthesis rate of *R. glacialis* leaves showed that it was on average 5.7 mg CO_2 dm^{-2} h^{-1} (range of 4.0–6.9 mg CO_2 dm^{-2} h^{-1}) under natural conditions and approximately 17.7 mg CO_2 dm^{-2} h^{-1} (range 9.2–23.7 mg CO_2 dm^{-2} h^{-1}) in the nursery. These data suggested that plants under nursery conditions may potentially accumulate significantly more organic matter; however, this was not observed. We speculate that for *R. glacialis*, nursery conditions were conducive for increasing the rate of photosynthesis but unfavorable for the accumulation of organic matter, and energy fixed by photosynthesis (APT, NADPH) was used rather for protective mechanisms then for biomass growth. This conclusion is supported by the results of an experiment that was carried out at PABGI. The introduction of *R. glacialis* into culture dates back to 1966, when adult plants were transplanted from natural conditions to the nursery of the PABGI. In 1970, however, all the plants died for unknown reasons. In the plants transplanted in 1971, flowering, fruiting, and in some years even self-sowing were observed, but, as a rule, plants died at the initial stages of development (by the end of the first – the beginning of the second year of life). As a result, low germination capacity, slow development, and high mortality of individuals were apparent.

In the Khibiny, *R. glacialis* is a rather rare plant. Additionally, mining contributes to the decrease of its populations. Low seed germination, slow development, and high mortality of individual plants impede the introduction of this species into culture. Despite its ornamental value, *R. glacialis* can hardly be used for landscaping. The main ecological conditions for the vigorous growth of the species are: humus-poor soils, relatively strong overwetting owing to excess of cold melt water in places of late snowmelt, and weak competition. It was shown that the cultivation of *R. glacialis* in the PABGI nurseries resulted in a gradual change of morphometric indicators of assimilating organs (decrease in leaf weight and area), reduction of plant habitus, gradual loss of the flowering phase, and quantitative rearrangement of leaf mesostructure. The results of the study, therefore, indicate that the requirements of this species do not correspond to the conditions of the PABGI nursery. The experiments with manipulated warming climate conducted in Norway revealed that the introduction of *R. glacialis* into the habitat of other species with better competitive capacity would lead to a decrease in their population density (Totland and Alatalo 2002). We observed this phenomenon when *R. glacialis* was introduced into the PABGI culture.

With this study, we showed that *R. glacialis* species had a low level of genetic diversity and a limited adaptive potential in the extreme eastern edge of its range in Russia (Kola Peninsula), as evidenced by numerous experiments on acclimatization of *R. glacialis* under nursery conditions in the Khibiny Mountains.

References

Alsos, I. G., Ehrich, D., Thuiller, W., Eidese, P. B., Tribsch, A., Schonswetter, P., Lagaye, C., Taberlet, P. and Brochmann, C. (2012): Genetic consequences of climate change for northern plants. *Proceedings of the Royal Society B: Biological Sciences*, 279: 2042-2051. doi: 10.1098/rspb.2011.2363

Andreeva, V. N. (1985): Seed productivity and germination of seeds of *Beckwithia glacialis* (L.) A. et D. Love – a rare species of flora of the USSR. *In*: Botanical research beyond the Arctic Circle. Apatity: KFAN USSR, pp. 48–58. (In Russian).

Bonin, A., Nicole, F., Pompanon, F., Miaud, C. and Taberlet, P. (2007): Population adaptive index: a new method to help measure intraspecific genetic diversity and prioritize populations for conservation. *Conservation Biology,* (21): 697-708. doi: 10.1111/j.1523-1739.2007.00685.x

Hughes, A. R., Inouye, B. D., Johnson, M. T. J., Underwood, N. and Vellend, M. (2008): Ecological consequences of genetic diversity. *Ecology Letters*, (11): 609-623. doi: 10.1111/j.1461-0248.2008.01179.x

Ivanov, L. A., Ivanova, L. A., Ronzhina, D. A. and Yudina, P. K (2013): Changes in the content of chlorophylls and carotenoids in the leaves of steppe plants along the latitudinal gradient in the Southern Urals. *Russian Journal of Plant Physiology*, 60(6): 812-820. doi: 10.1134/s1021443713050075. (In Russian).

Ivanov, L. A., Ronzhina, D. A., Ivanova, L. A., Chechulin, M. L., Belousov, I. V., Gunin, P. D. and Pyankov, V. I. (2004): Structural and functional features of adaptation of Gobi plants to climate aridization. *Arid Ecosystems*, 10(24-25): 90-102.

Ivanova, L. A. (2014): Adaptive features of the leaf structure of plants of different ecological groups. *Ecology*, 2: 109-118. doi: 10.7868/S0367059714020024. (In Russian).

Ivanter, E. V., Korosov, A. V. (2010): Elementary biometrics. Petrozavodsk: Petrozavodsk State University, 104 p. (In Russian).

Koroleva, N. E., Kopeina, E. I. (2020): Rare and endangered vegetation and vascular plants in canyon “Gorodskaya shchel’ (Town Crack)” in southern part of Khibiny Mountains (Murmansk Region, Russia). *Arctic Environmental Research*, 20(1): 17-28. doi: 10.3897/issn2541-8416.2020.20.1.17

Kudryavtseva, O. V., Shmakova, N. Yu., and Kuzmin, A. V. (2001): Quantitative-anatomical and productive characteristics of assimilating organs of plants-dominants of mountain tundra Khibiny. *Botanicheskii Zhurnal*, 86(9): 108-115. (In Russian).

Lichtenthaler, H. K., Wellburn, A. R. (1983): Determination of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochemical Society Transactions*, 11(5): 591-592.

Maslova, T. G., Popova, I. A. (1993): Adaptive properties of the plant pigment systems. *Photosynthetica*, 29(2): 195-203.

Mokronosov, A. T. (1978): Mesostructure and functional activity of the photosynthetic apparatus. *In*: A. T. Mokronosov (ed): Mesostructure and functional activity of the photosynthetic apparatus. Sverdlovsk. pp. 5–30. (In Russian).

Mokronosov, A. T., Borzenkova, R. A. (1978): Method of quantitative estimate of the structure and functional activity of photosynthetic tissues and organs. *Transactions Applied Botany, Genetics and Breeding*. 61(3): 119-133. (In Russian).

Mokronosov, A. T. (1981): Ontogenetic aspects of photosynthesis. Moscow: Nauka, 198 p. (In Russian).

Nakonechnaya, O. V., Gafitskaya, I. V., Burkovskaya, E. V., Khrolenko, Y. A., Grishchenko, O. V., Zhuravlev, Y. N., Subbotin, E. P. and Kulchin, Y. N. (2019): Effect of light intensity on the morphogenesis of *Stevia rebaudiana* under in vitro conditions. *Russian Journal of Plant Physiology*, 66(4): 304-312. doi: 10.1134/S0015330319040092(In Russian).

Parmesan, C. (2006): Ecological and evolutionary responses to recent climate change. *Annual Review of Ecology, Evolution, and Systematics*, (37): 637-669. doi: 10.1146/annurev.ecolsys.37.091305.110100

RAMAZANOVA, Z. R., ASADULAEV, Z. M. (2012): Morpho-anatomical variations of rod's systems of *Celtis caucasica* Willd. in Mahachkala. *In*: Biological reclamation and monitoring of disturbed land. Ekaterinburg: Ural University, pp. 209–214. (In Russian).

SAUTKINA, T. A., POLIKSENOVA, V. D. (2011): Plant morphology. Minsk, 24 p. (In Russian).

SEMKO, A. P. (1989): Heat and moisture regime for the growth and development of wild and introduced plants in the central part of the Kola Peninsula. Apatity: KFAN USSR, 30 p. (In Russian).

STREB, P., SHANG, W., FEIERABEND, J. and BLIGNY, R. (1998): Divergent strategies of photoprotection in high-mountain plants. *Planta*, 207: 313-324.

STREB, P., AUBERT, S., GOUT, E. and BLIGNY, R. (2003): Reversibility of cold- and light-stress tolerance and accompanying changes of metabolite and antioxidant levels in the two high mountain plant species *Soldanella alpina* and *Ranunculis glacialis*. *Journal of Experimental Botany*, 54(381): 405-418. doi: 10.1093/jxb/erg048

STREB, P., JOSSE, E-M., GALLOUËT, E., BAPTIST, F., KUNTZ, M., and CORNIC, G. (2005): Evidence for alternative electron sinks to photosynthetic carbon assimilation in the high mountain plant species *Ranunculus glacialis*. *Plant, Cell and Environment*, 28(9): 1123-1135. doi: 10.1111/j.1365-3040.2005.01350.x

TOTLAND, O., ALATALO, J. M. (2002): Effects of temperature and date of snowmelt on growth, reproduction, and flowering phenology in the arctic/alpine herb, *Ranunculus glacialis*. *Oecologia*, 133: 168-175. doi: 10.1007/s00442-002-1028-z

VASILEVSKAYA, N. V. (2007): Polyvariance of plant ontogenetic processes in conditions of high latitudes. Murmansk: MSPU, 231 p. (In Russian).

VASFILOV, S. P. (2011): The analysis of the causes of variability of the relationship between leaf dry mass and area in plants. *Journal of General Biology*, 72(6): 436-454. (In Russian).

WAGNER, J., STEINACHER, G. and LADINIG, U. (2010): *Ranunculus glacialis* L.: Successful reproduction at the altitudinal limits of higher plant life. *Protoplasma,* (243): 117-128. doi: 10.1007/s00709-009-0104-1

ZYUZIN, YU. L. (2006): Stern face of the Khibiny. Murmansk: Advertising polygraphy, 236 p. (In Russian).

Web sources / Other sources

[1] Red Data Book of Murmansk Region (2014), Kemerovo: Aziya-Print, 578 p. (In Russian).