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Ecologo-Biological Characteristics and Pigment Contents of *Saxifraga* Species with Different Growth Forms on West Spitsbergen

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Abstract: Eleven *Saxifraga* species with different growth forms from the Arctic tundra of West Spitsbergen have been characterized by their content of photosynthetic pigments (chlorophyll *a* and *b* (Chl), carotenoids (Car)), the Chl a/b and Chl/Car ratios, the size of light-harvesting complex of PS II (LHC-II), total flavonoid content and total leaf nitrogen content. The total Chl content varied from 0.39 to 1.14 mg/g FW; the total carotenoid content varied from 0.28 to 0.10 mg/g FW; the Chl *a*/*b* ratio was in the range of 2.2 to 3.5; the Chl/Car ratio was in the range of 2.7 to 4.7; the LHC-II size varied from 50 to 69 %. The investigated species represented three growth forms - rosette, semirosette and cushion. It was shown that the average photosynthetic pigment content increases from cushion to semirosette and to rosette plants. It is suggested that the main adaptation of photosynthetic apparatus to extreme conditions in saxifrages with different growth forms is the decrease of Chl content on species level that ensures high functional activity of plants.

Key words: Genus *Saxifraga* · Life forms · Pigments · Light-harvesting complex · Arctic · Spitsbergen

INTRODUCTION

In recent years there has been increasing interest to the regularities and mechanisms of adaptations that plants develop at high latitudes. A number of taxa of generic rank demonstrate a wide range of adaptations to the high latitude climate [1]. Taxa that are characterized by great variety of growth forms attract most interest. One of them is the genus *Saxifraga,* the largest one in the family *Saxifragaceae*, containing about 450 species of the Arctic and northern hemisphere mountain flora [2]. There are 14 saxifrages in the flora of Spitsbergen [3].

Patterns of geographic distribution of saxifrages, taxonomic isolation of the genus and division to sections, relationships of which are not always clear testify to the antiquity of the genus [4]. The genus is represented by plants with different growth forms from primitive creeping cushion to evolutionarily advanced real rosette. Saxifrages are capable of reproducing both sexually (seeds) and asexually (vivipary, bulbils, above-ground runners (stolons)). It ensures their reproduction under extreme Arctic conditions. The relation between plant functional status and ways of structural adaptation (various growth forms) in the Arctic has not been studied.

The aim of this work was to investigate the differences in pigment contents and composition in *Saxifraga* species with different growth forms that grow on West Spitsbergen.

MATERIALS AND METHODS

The work was carried out in a high Arctic tundra ecosystem in the interior fiord area of West Spitsbergen that is characterized by closed canopy and high diversity of vascular plants. The research was conducted in several areas of West Spitsbergen between 2007 and 2011. At that latitude the polar day lasts from April 19 to August 24. Above-zero temperatures are generally kept from June 5 to September 18. The warmest month is July with average temperature of 8°C. The average annual rainfall is 563 mm.

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Most precipitation occurs during winter [5]. Short growing season (40-70 days) is determined by the Arctic climate, particularly by the length of snow-free period.

Leaf samples were taken from mature leaves of 2-5 flowering plants, fixed and conserved in 96% ethanol. Chl *a*, Chl *b* and total carotenoids content was determined spectrophotometrically (SF-26, Russia). The size of LHC-II was calculated as the percentage of Chl in LHC-II assuming that almost all Chl *b* is in LHC and the Chl *a/b* ratio is 1.2 [6-9]. Total leaf nitrogen was determined in leaf samples dried at 60ºC by Kjeldahl method. It is known that photosynthetic capacity of leaves is related to the nitrogen content primarily because the proteins of the Calvin cycle and thylakoids contain the most of leaf nitrogen [10-11]. This relation was described for a number of arctic plants including saxifrages [12]. The total flavonoid content in leaves was determined spectrophotometrically at 410 nm [13].

Totally 11 *Saxifraga* species were studied: *S. hieracifolia, S. foliolosa, S. nivalis, S. tenuis, S. rivularis, S. cernua, S. hirculus,* S. platysepala, S. aizoides, S. îppositifolia, S. cespitosa.

RESULTS AND DISCUSSION

In the studied saxifrages the total Chl content varied from 0.39 to 1.14 mg/g FW, the total carotenoids content varied from 0.10 to 0.28 mg/g FW, the Chl *a/b* ratio was in the range of 2.2 to 3.5, the Chl/Car ratio was in the range of 2.7 to 4.7, the size of the LHC II varied from 50 to 69% (Table 1). These values are comparable to those obtained for the content of Chl (0.39-2.57 mg/g FW) and Car (0.10 to 0.86 mg/g FW) when 91 species of vascular plants from West Spitsbergen were analyzed.

Rosette. In saxifrages that form rosettes the Chl content varies in a widest range - from 0.51 to 1.14 mg/g FW, the Car content varies from 0.19 to 0.25 mg/g FW, the Chl *a/b* ratio is in the range of 2.2 to 3.2, the Chl/Car ratio is in the range of 2.7 to 4.7, the size of LHC-II varies from 52 to 69% (Table 1). The species that form rosettes belong to one subgenus and section *Micranthes* [2, 14, 15]. They are all known as evergreen, circumpolar, arctalpine plants with short rhizomes [16].

S. hieracifolia is a polyploid species that is characterized by considerable variability of a number of features (size, leaf form, indumentums, flower colour), and stability of the main morphological features. It is regarded as an aggregate species. This is the largest in terms of size *Saxifraga* species in the Arctic tundra. In Spitsbergen it grows in a limited number of ecotopes with high soil moisture content and well-developed moss growth. Among studied saxifrages *S. hieracifolia* has the highest Chl content (Table 1). Low Chl *a/b* ratio indicates shade tolerance of photosynthetic apparatus (PA). High Chl/Car ratio testifies to smaller protective role of Car compared to flavonoids, which content is relatively high.

Table 1: Content of photosynthetic pigments, total nitrogen and flavonoids in *Saxifraga* species on West Spitsbergen

Species	Dry weight/Fresh weight (DW/FW), %	Chl *a+b* content, mg/g FW	Chl *a/b*	Carotenoid content, mg/g FW	Chl/Car	Chl in LHC-II/Total Chl, %	Total nitrogen content, % of DW	Flavonoid content, % of DW
Rosette								
Saxifraga foliolosa R. Br.	24	0.51±0.03	2.7	0.19±0.01	2.7	60	2.88	0.85
S. tenuis (Wahlenb.) H. Sm.	28	0.95±0.05	3.0	0.25±0.02	3.8	55	ND	ND
S. nivalis L.	23	0.98±0.09	3.2	0.24±0.02	4.1	52	2.48	5.90
S. hieracifolia Waldst. andKit.	22	1.14±0.11	2.2	0.24±0.02	4.7	69	2.17	6.47
Semi-rosette								
S. platysepala (Trautv.) Tolm.	21	0.48±0.04	2.8	0.18±0.01	2.7	60	ND	ND
S. cernua L.	14	0.52±0.05	2.9	0.16±0.01	3.2	55	1.73	5.54
S. rivularis L.	13	0.66±0.04	3.5	0.19±0.02	3.5	50	2.40	4.69
S. hirculus L.	20	0.89±0.05	3.2	0.28±0.02	3.2	52	2.61	9.05
Cushion								
S. cespitosa L.	16	0.39±0.03	2.5	0.10±0.01	3.9	62	1.46	5.67
S. aizoides L.	21	0.65±0.06	2.6	0.18±0.01	3.6	61	1.91	6.63
S. oppositifolia L.	26	0.66±0.06	2.7	0.19±0.01	3.5	60	1.50	6.26

Note. ND - no data.

S. foliolosa is a polyploid species with great number of ecotypes that differ in plant size, degree of inflorescence branching, number and position of flowers. This plant is a specialist of wet sites. It usually grows on sedge and cotton grass bogs and by the edge of small streams. Seed reproduction was hardly recorded for *S. foliolosa.* Vegetative reproduction in the form of vivipary is common as the additional adaptation to environmental conditions [4]. On the Westen Spitsbergen this species is represented by the ecotype with small plant size, oligophyllous rosette, solitary stem and sometimes bulbils instead of flowers. The species is characterized by low total Chl content and relatively high Car content (Table 1).

The values of pigment ratios testify to the shade tolerance of PA. The maximum value of the total nitrogen content in this species is proportionate to those obtained for larger species of this family, which may testify to high functional activity of the species and high protection of PA that is likely to be provided by Car as the minimal content of flavonoids indicates their minor role in PA protection.

S. nivalis and *S. tenuis* are closely related species of wet habitats that are common in phenological phase duration. *S. nivalis* is a polymorphic, large, highly variable species in terms of height, inflorescence shape, flower number, leaf shape and indumentum. It prefers gravel substratum in dry as well as in wet habitats. All differences in habitus of *S. nivalis* are determined by ecotopes. *S. tenuis* is a smaller species. It is chionophilous plant that grows in snow patch group in mountain localities. It originates from high mountains of the Neogene Arctic which are under the influence of prolonged snow pack. Both species have similar pigment content and composition (Table 1) that are typical for sun-loving plants. The relatively high total nitrogen content in *S. nivalis* (Table 1) testifies to high plant functional activity. Protection of PA is provided by both Car and flavonoids.

Thus, saxifrages that form rosettes (evolutionarily advanced growth form) are characterized by a wide range of photosynthetic pigment content and high total Chl content.

Semirosette. Saxifrages that form semirosettes belong to two subgenera (*Saxifraga*, *Hirculus*) and three sections (*Saxifraga, Ciliatae, Flagellares*).

In this plant group the Chl content varies within a narrower range (0.48-0.89 mg/g FW) compared to rosette plants. The Car content varies from 0.16 to 0.28 mg/g FW, the Chl *a/b* ratio is in the range of 2.8 to 3.5, the Chl/Car ratio is in the range of 2.7 to 3.5, the size of LHC-II varies from 50 to 60% (Table 1).

S. rivularis and *S. cernua* belong to the same subgenus and section.

S. rivularis is an amphi-Atlantic meta-arctic hemi-chionophilous species that specializes in moist and wet, rocky places. It is represented by two forms that differ in colour and size. It reproduces by seed and rooting stolons. Among studied species *S. rivularis* has medium values of photosynthetic pigment and flavonoid content, but relatively high total nitrogen content (Table 1), which may testify to high functional activity of PA. The Chl *a/b* ratio indicates that it is a sun-loving species. PA protection is provided by Car and flavonoids.

S. cernua is a circumpolar arctalpine polymorphous species with a large number of forms. It is widespread in the Arctic and grows in a wide variety of habitats. One form that is described as a new species *S. svalbardensis* is found on Spitsbergen [3]. *S. cernua* is adapted to the extreme environmental conditions due to efficient vegetative reproduction by bulbils (vivipary), contribution of which increases further North. *S. cernua* differs from the previous species in pigment composition. It has higher Chl *b*, Car and flavonoid content and lower total nitrogen content (Table 1). It testifies to lower functional activity and vulnerability of PA which may stimulate frequent occurrence of vegetative reproduction.

S. hirculus and *S. platysepala* belong to one subgenus, but different sections.

S. hirculus is a circumpolar Arctic-Boreal (the only Arctic-Boreal species among the studied saxifrages) polymorphous species that grows in wet places in all tundra types and specializes in areas with deep snow. Its wide geographic distribution including different climatic zones (from western Siberian forest-steppe to high Arctic tundra) resulted in a large variety of ecobiomorphs. The peculiarity of its origin is that it is not connected with mountain ecotopes. Under Arctic conditions it reproduces mainly asexually by stolons or rhizomes. Among studied semirosette saxifrages *S. hirculus* is characterized by the highest Chl content. Pigment ratios are rather common, which indicates that the species is well adapted to its environment. *S. hirculus* has the high total nitrogen content that testifies to high functional activity of PA. The high amount of flavonoids as protective pigments is likely to be due to partially boreal origin.

S. platysepala is a circumpolar Arctic species that belongs to a small group of polyploid flowering plants extending their range to the edge of the polar lands. Extreme environmental conditions with limited summer warmth determine the ability of plants to form long stolons terminating in a tiny rosette that attaches to the substrate by adventitious roots and may develop

flowering stem. The Chl content in *S. platysepala* leaves is low compared to other *Saxifraga* species. The values of pigment ratios indicate that the species is shade-tolerant. Total Chl content in stolons and young rosette leaves is correspondingly lower by 10 and 30% compared to that in leaves of adult plants. The Chl *a/b* ratio in these organs is also lower, which indicates greater contribution of Chl *b* to plant pigment complex. Similar low values of total Chl content were found in leaves of *S. platysepala* (0.5 mg/g FW) on Wrangel Island [17]. The investment of more resources to asexual reproduction (stolos, rhizome) than to sexual reproduction (flowers and seeds) is a functional advantage of the species that ensures its extension to the North [18].

Thus saxifrages that form semirosettes appeared to be heterogeneous in terms of morphologic, systematic and physiological indices. Their content of pigments is lower than in rosette saxifrages. The significance of Car and Chl *b* in PA protection is similar in rosette and semirosette plants.

Cushion. *S. aizoides* and *S. îppositifolia* belong to one subgenus, but different sections. *S. cespitosa* belongs to another subgenus and section. This group of species is characterized by the narrowest range of the Chl content (0.39-0.66 mg/g FW) among studied species, relatively constant Chl *a/b* ratio (2.5-2.7) and values of LHC-II (60-62%) (Table 1). The Chl/Car ratio is in the range of 3.5-3.9.

S. cespitosa (cushion) is a circumpolar arctalpine species that grows in high arctic tundra. It forms large cushions with very short flowering stems, with tap root or adventitious tap root system. *S. caespitosa* is a polymorphous species with numerous forms that differ in flower size and inflorescence length. It is spread in all northern subzones (from shady rocks in northern taiga to polar deserts) and various ecotopes. The species is characterized by the lowest values of the Chl and Car content among all studied saxifrages. The higher proportion of Chl *b* indicates shade tolerance of the species and the relatively low content of Car testifies to their weak contribution to PA protection in contrast to flavonoids. Low total nitrogen content indicates low protein content and likely testifies to lowest functional activity of PA among studied saxifrages. However, large size of cushions and long photosynthetic activity of the species during the growing season ensures large biomass accumulation and energetic maintenance of seed formation and maturation.

S. aizoides is an amphi-Atlantic (can be found in eastern north America and Europe) arctalpine (loose cushion) species. The species is an obligate calciphyte that prefers dry rather than wet habitats and often can be found in rocky soils. The species is characterized by the medium Chl content compared to other studied saxifrages and relatively high Chl *b* content due to the large size of LHC-II (Table 1). Relatively low functional activity indicated by low total nitrogen content and relatively high flavonoid content among studied saxifrages testifies to the vulnerability of plant photoassimilation system and requirement of its special protection.

S. îppositifolia is a circumpolar arctalpine species (creeping cushion) that is widespread in the alpine regions of Europe, Asia and Northern America. In the Arctic it reaches the edge of the polar lands. This species is regarded as an aggregate complex that is characterized by high variability and flexibility in the Arctic. The interest to this species is due to its wide distribution and various growth forms under different ecological conditions. Two forms can be distinguished: the cushion form (C-form) and prostrate form (P-form). The forms differ in morphology, habitats, growing season duration, number of reproductive organs, asexual/sexual reproduction ratio, ability to form rhizomes, competitive strength, rate of expansion and flexibility level. According to our data, the total Chl content was 0.63 mg/g FW for C-form plants and 0.70 mg/g FW for P-form plants. The Car content was 0.17 and 0.20 mg/g FW respectively. Plants with different growth forms did not differ in pigment ratios. According to the data of Kume *et al*. [18] C-form and P-form plants of *S. oppositifolia* had similar net photosynthesis. Relatively low total nitrogen content (1,5% according to our data) may indicate relatively low rate of net photosynthesis of the species. It is supported by the data of Maruoka *et al*. [12] on low nitrogen content (1.83%) in the leaves of *S. îppositifolia* (Ny-Ålesund), which corresponded to low photosynthetic rate.

Thus, saxifrages that form cushions are characterized by the narrowest range of Chl content among studied growth forms. The Chl *a/b* ratio indicates their shade tolerance. Low total nitrogen content and relatively high flavonoid content may testify to low functional activity of PA and the necessity of its protection in these species.

The species ecological amplitude width is assessed by its activity. According to Koroleva [5] *S. cespitosa* (cushion)*, S. rivulari and S. cernua* (semirosette), and *S. foliolosa* (rosette) are to be included in the group of the most active species of arctic tundra and polar desert ecosystems. These species are characterized by low or medium level of Chl content that supports our earlier suggestion that species with low or medium pigment content are more active in forming mountain tundra plant associations [19].

DISCUSSION

The analysis has shown that there is a relation between the growth form and photosynthetic pigment content in *Saxifraga* species. The general trend is the increase of the average content of photosynthetic pigments (chlorophylls and carotenoids) from cushion plants to semirosette and then to rosette plants. Species having evolutionarily more advanced morphological structure are characterized by higher content of photosynthetic pigments. It is also known that Chl content decreases with the increase of severity of conditions, i.e. with the spreading of plants to the North [20]. Plants with primitive cushion growth form have lowest pigment content. Similar dependence was observed between the growth form and total nitrogen content that enables to suppose that the same relation exists between the growth form and photosynthetic rate in *Saxifraga* species. The Chl *a/b* and Chl/Car ratios and respectively the size of LHC-II are mainly determined by species ecology and depend on ecological conditions and the degree of species light demand or shade tolerance. Shade tolerance of cushion plants is determined by their similar morphology. The LHC-II size in these plants is usually stable (about 60%).

Obtained data have shown that Chl content values in different morphs of *S. oppositifolia* is a genetically determined feature. It was also confirmed by the data obtained for different ecomorphs. Our data on total nitrogen content in the leaves of *S. oppositifolia* that grows on West Spitsbergen support the suggestion to possible use of this index in the assessment of plant response to Arctic climate changes [12, 21, 22].

The investigation revealed that due to their flexibility saxifrages on West Spitsbergen are presented by a wide variety of growth forms that occupy a wide range of ecotopes. The main adaptation of PA to extreme conditions in saxifrages is the decrease of Chl content on species level that ensures high functional activity of plants with reduced investment of resources (including antioxidant resources) to its maintenance. Intraspecific genetic and ecological variation is common phenomenon for many arctic plants [23]. The variation on the level of morphology and flexibility provides additional arguments to support the strategy of Arctic species conservation.

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