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Eco-physiological peculiarities of *Stellaria humifusa* in West Spitsbergen

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Abstract

In the present paper, the results of the research of biometric parameters and functional peculiarities of photosynthetic apparatus of *Stellaria humifusa* in West Spitsbergen are discussed. The study showed that the largest proportion of the mat is composed of a brown layer of dead leaves covering the assimilative organs (about 70%). Green leaves and shoots accounted for about 20%, whereas generative organs – for about 10% of the mat mass. The values of the maximum photochemical quantum yield of PS II (F_V/F_M) in all the studied plants were lower than the optimal values (0.83), which suggests that the plants are exposed to stress factors. The low values of chlorophyll and carotenoid content and relatively high values of the light-harvesting complex (70-80%), the sharp decrease in the quantum yield of PS II (Φ_{PSII}) entailed by a marginal increase in PPFD indicative of a sufficiently high of photochemical activity within the range of low and medium values of light. It also suggests that *Stellaria humifusa* is well-adapted to the shaded conditions created in the mat. Besides, a considerable decrease the maximum fluorescence yield of a light-adapted leaf (F_M') at increasing PPFD was observed, which suggests that *Stellaria humifusa* has a well-developed mechanism of energy dissipation via the non-photochemical (NPQ) pathway. The rapid development of NPQ gives reason to assume that, under high light conditions, non-photochemical quenching is likely to serve as the main mechanism for preventing the photodamage of the photosynthetic apparatus. Thus, it was shown that the photosynthetic apparatus of *Stellaria humifusa* works efficiently under the conditions created in the mat, and the thick layer of dead leaves covering the assimilative organs, on the one hand, protects them from excessive light, and on the other hand, absorbs thermal energy, which raises temperature of the local habitat.

Key words: Chlorophyll fluorescence, photosynthetic apparatus adaptation, photosynthetic pigments, mat morphological peculiarities

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Introduction

The Arctic region is characterized by extreme environmental conditions. The main adverse factors exerting a negative impact on plants are low air and soil temperatures, the low availability of nutrients in the soil, constant winds, a short growing season which coincides with the polar day and results in round-the-clock solar radiation (Callaghan et al. 2004, Li et al. 2013).

In view of this, the study of the biological peculiarities of certain species growing in the Arctic region is of special interest, since, under these extreme conditions, living organisms use a wide range of adaptations to environmental factors. Those adaptations manifest themselves at the anatomical, morphological, biochemical and physiological levels (Lütz et al. 2012). In recent years, in connection with the problem of climate change, the Arctic region has been attracting increased interest of researchers. The polar regions are the most vulnerable to global warming, compared to the rest of the Earth surface ([1] - IPCC 2013). At the same time, the phytocenosis in high latitudes is characterized by a more distinct manifestation of the dependence between the vital functions of living organisms and climatic factors (Shmakova and Markovskaya 2010). Thus, revealing the mechanisms allowing plants to adapt to the environmental conditions of the Arctic region is of great importance.

The photosynthetic apparatus and its reaction to the exposure to various factors are a reliable source of information on the well-being of the plant and its capacity for adaptation to the changing environmental conditions (Goltsev et al. 2014). In its turn, chlorophyll fluorescence is a reliable source of information on the functional state of the photosynthetic apparatus (Goltsev et al. 2014). Little is known about chlorophyll fluorescence of plants growing in Spitsbergen (Barták et al. 2012, Li et al. 2013).

Stellaria humifusa Rottb. (Caryophyllaceae) is a perennial plant which is abundant in Spitsbergen and mostly inhabits salt marshes and in moisty coastal lands. *Stellaria humifusa* has small leaves (2-4 mm) and thin ramulous shoots. The life form is a cushion plant and plants form large, low-growing mats. Among the peculiarities of this species it is important to note the formation of single-species mats with an outer brown layer of dead leaves (the dead leaves are preserved throughout the winter and often all through the next season). Under these layers, green leaves can be found, which occupy the whole internal structure of the mat (Rønning 1996).

In the literature, there is a detailed description of the biological peculiarities of this species. However, studies of its physiology are scarce (Markovskaya and Shmakova 2012, Markovskaya and Shmakova 2017, Markovskaya et al. 2017). The research objective included biometrical studies and research functional peculiarities of photosynthetic apparatus of *Stellaria humifusa* growing in West Spitsbergen.

Material and Methods

The study was conducted in the Arctic tundra zone in the vicinity of the township of Barentsburg (78° 04′ N, 14° 28′ E) in West Spitsbergen in July 2015 and 2018. At the latitude of Barentsburg, from April 19 to August 24 it is the time of a polar day. The mean annual temperature is +5.8°C, the average temperature of the warmest month (July) is +8.0°C. The average annual precipitation is 563 mm. Throughout the year, a high relative humidity is observed. The normal value of this parameter is 78%.

In summer, fogs often occur (Koroleva et al. 2008). These climatic features determine the short growing season – no more than 40-70 days (Ronning 1996). In July 2015 and 2018, the average daily air temperature was +7.6°C and +6.1°C, the relative humidity of the air was 71% and 85%, respectively.

The subject of the study is *Stellaria humifusa* Rottb., Caryophyllaceae. For the purposes of the research, plants were sampled in the coastal zone on the moisty rocky substrate with a slight infusion of clayey soil, where they formed large vegetation single-species mats (up to 10 m in diameter). At the moment of the study, the plants were blossoming.

Measurement of biometric parameters: The mat thickness was measured with a ruler. To determine the biomass distribution in the mat by fractions (dead leaves, green leaves and shoots, generative organs) they were weighed. The work was performed on the largest mat ($1.5\times1.2m^2$) from different parts of which 3 samples (10×10 cm^2) were taken.

Measurement of plastid pigment: To study the content of pigments, 3 replications were taken from each sample (each repetition included 10 leaves). Chlorophylls (Chl *a* and *b*) and carotenoids (Car) contents were estimated in ethanol extracts spectrophotometrically with an UV–1800 (Shimadzu, Japan) at corresponding absorption maxima. The proportion of Chl in the light-harvesting complex (LHC) was calculated, assuming that the whole Chl *b* is present in the LHC, where the Chl *a/b* ratio is 1.2 (Lichtenthaler and Wellburn 1983).

Determination of chlorophyll fluorescence: Small fragments of the mat were taken, from which green shoots were sampled. In total, 30 plants were sampled. The measurements were taken on the second pair leaves from the shoot base. To measure the chlorophyll fluorescence the fluorimeter with a pulse-modulated light source (JUNIOR-PAM, Walz, Germany) was used. The chlorophyll fluorescence parameters were registered after 30-min. darkness adaptation. The following parameters were determined: basic fluorescence (F_0), maximal fluorescence (F_M) and variable fluorescence (F_V) (the saturating impulse PPFD being 10 000 μmol m^{-2} s^{-1}), the maximum photochemical quantum yield of PS II (F_V/F_M).

The rapid light curves (RLCs) were obtained using the WinControl software. The maximum fluorescence yield of a light-adapted leaf (F_M'), the relative electron transport rate (ETR), the quantum yield of PS II (Φ_{PSII}), the coefficients of non-photochemical fluorescence quenching (NPQ) were registered at PAR being 66, 90, 125, 190, 285, 420, 625 and 820 μmol m^{-2} s^{-1}.

Results

The mat thickness varied in the range of 2.5-4 cm. The measurements of the biomass fractions proportions. showed that the greater part of the mat (about 70%) is composed of a brown layer of dead leaves, which covers the assimilative organs. Green leaves and shoots account for about 20%, and generative organs – for about 10% of the mat mass.

Chlorophyll content varied in the range of 2.4-6.7 mg g^{-1} of dry mass, carotenoids content: 0.45-1.29 mg g^{-1} of dry mass, on average amounting to 4.01 mg g^{-1} of dry mass for chlorophylls content and 0.84 mg g^{-1} for carotenoids. The chlorophyll *a* to chlorophyll *b* ratio was within the range of 1.9-2.9, the ratio of green pigments to yellow ones was 4.1-5.3, the size of the light-harvesting complex varied from 56 to 76%.

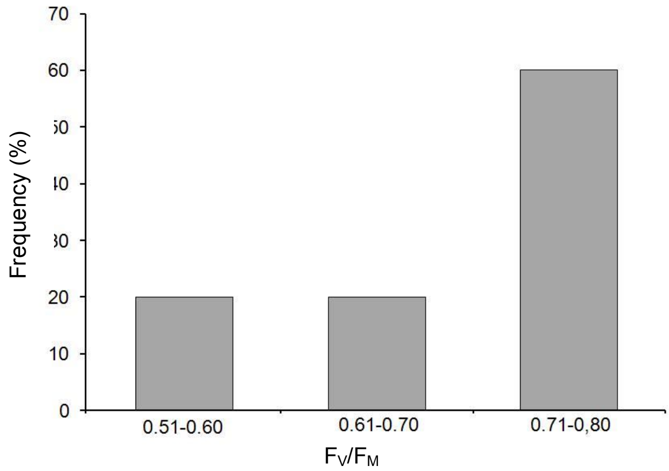

Fig. 1. The distribution of the studied plants on values of the maximum photochemical quantum yield of PS II (F_V/F_M).

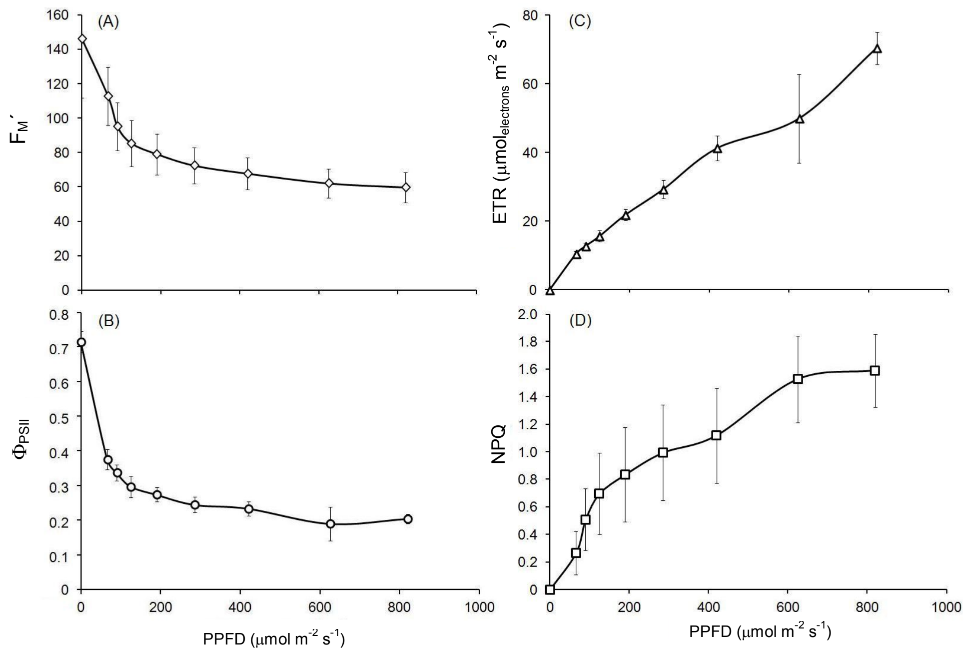

Fig. 2. Parameters of rapid light curves: (A) maximum fluorescence yield of a light-adapted leaf (F_M'), (B) the quantum yield of PS II (Φ_{PSII}), (C) the relative electron transport rate (ETR), (D) the coefficients of non-photochemical fluorescence quenching (NPQ) as a function of PPFD.

The study of the parameters of chlorophyll fluorescence showed that F_0 in the studied plants varied in the range from 22 to 52, F_M – from 56 to 182, F_V – from 34 to 144. The F_V/F_M values varied considerably in the studied plants (Fig. 1). 60% of all the studied plants had high values of F_V/F_M (over 0.7). In 20% of the studied plants, the values of this parameter varied within the range of 0.6-0.7, and in the remaining 20% of the plants – from 0.5 to 0.6. Lower F_V/F_M values were not observed.

The study of the light curves showed a rapid reduction F_M' with increasing irradiance (Fig. 2A). With PPFD increasing from 66 to 820 μmol m^{-2} s^{-1}, F_M' decreased by 47%. In the studied individuals, with increasing irradiance a rapid decrease in Φ_{PSII} occurs. At PPFD increasing up to 90 μmol m^{-2} s^{-1}, Φ_{PSII} decreases by 53%. Further increase in irradiance did not lead to significant changes in Φ_{PSII} (Fig. 2B). At increase PPFD from 190 to 285 μmol m^{-2} s^{-1}, the NPQ values increased, but still remained under 1.0. However, even at PPFD reaching 420 μmol m^{-2} s^{-1}, the NPQ values exceed 1.0, and they reach their peak (1.6) at PPFD equal 820 μmol m^{-2} s^{-1}. The ETR values went up with increasing PPFD. At the same time, we did not observe any decline in ETR at high PPFD values (Fig. 2C, D).

Discussion

The conducted research showed that the whole mat has a single-species structure, which includes the species *Stellaria humifusa*. There were also sporadic occurrences of *Puccinellia sp.* without blossoms. The mat thickness on average equaled 2.5–4 cm. The base of the mat was composed of a well-drained layer – humus horizon, in which the root system of this plant is found. This horizon is the metabolic by-product of the species, the organizational structure of which makes it possible to preserve all the leaf fall and re-use it actively in its lower part. The aboveground portion of the mat includes several components (the brown layer of last year's dead leaves, green leaves and shoots, and generative organs (flowers, buds and seeds). A large proportion of the brown mass covering the assimilative organs can play a key role in the protection of the assimilative apparatus of this species from excessive light. In order to test this hypothesis, a study of the condition and well-being of the plant's photosynthetic apparatus was conducted focusing on its chlorophyll fluorescence parameters.

The study of the chlorophyll fluorescence parameters showed that the values of F_0, F_M and F_V were relatively low (Markovskaya and Shmakova 2017). The low values of F_M and F_V are indicative of a low functional state of the photosynthetic apparatus, which can be associated with the fact that the plants are exposed to stress factors. At the same time, the low values of F_0 give evidence of an effective transfer of excitation energy between the pigments molecules in the light-harvesting complex (Goltsev et al. 2014).

As is well known, the F_V/F_M ratio can serve as an indicator of the photochemical activity of the photosynthetic apparatus (Maxwell and Johnson 2000). In all the studied plants, the values of this parameter were lower than the optimal values (0.83) (Björkman and Demming 1987), which suggests that the plants are exposed to stress factors. However, among the studied plants, in 60% of them the values of F_V/F_M were nearly optimal (over 0.7). The near-optimal values of F_V/F_M in the majority of the studied plants are indicative of the presence of well-functioning regulatory systems at the level of the photosynthetic apparatus. The observed decrease in this parameter can be associated with the inhibition of photosynthesis II and the reduction in the proportion of recreation centers, capable of restoring the terminal acceptor. The analysis revealed a high level of plants heterogeneity according to the functional condition of their photosynthetic apparatus (Fig. 1). A similar result was obtained in a number of flora species in Spitsbergen (*Pedicularis hirsuta, Salix polaris, Dryas octopetala* and others) (Markovskaya and Shmakova 2017). In this group, among widespread species, we can also find those with a limited habitat.

The drastic fall in Φ_{PSII} at an insignificant increase in PPFD is indicative of the adaptation of this species to low light conditions. Φ_{PSII} and ETR are parameters which are closely connected with photosynthetic rate (Maxwell and Johnson 2000). As compared to other species growing in Spitsbergen, *Stellaria humifusa* is characterized by medium values of Φ_{PSII} and ETR (Muraoka et al. 2008, Barták et al. 2012, Markovskaya and Shmakova 2017).

For *Stellaria humifusa*, a significant decline in F_M' at increasing PPFD was observed (Fig. 2). This suggests that this species has a well-developed mechanism of energy dissipation via the NPQ (Ralph and Gademann 2005). We also observed a rapid rise in NPQ along with an increase in PAR. The rapid increase in NPQ suggests that, under high light conditions, non-photochemical quenching can serve as the main mechanism which prevents the photodamage of the photosynthetic apparatus (Krause and Weis 1991, Horton et al. 1996).

These findings provide a clear explanation for the non-typical growing conditions of the plants of this species, which, in the setting of this particular ecotope, are protected from the bright sunshine by a layer of dark died leaves. This layer, on the one hand, covers the plants from direct sunlight, and on the other hand, it absorbs thermal energy, raising the species habitat temperature. The analysis of the data on chlorophyll fluorescence showed that the photosynthetic apparatus of *Stellaria humifusa* works effectively under low light conditions, which are typical of this habitat.

Stellaria humifusa belongs to vascular plants of West Spitsbergen with a low plastid pigments content. The plastid pigments content does not depend on the particular habitat of the species in West Spitsbergen, which is confirmed by our long-term data (Markovskaya and Shmakova 2017). Data on the content of photosynthetic pigments and their ratio suggest that *Stellaria humifusa* is well-adapted to the shaded conditions created in the mat. The low chlorophyll content is indicative of a potentially low photosynthetic rate (Buttery and Buzzell 1977) and, consequently, a low biological productivity. At the same time, the analysis of the chlorophyll fluorescence parameters indicates a sufficiently high capacity for photochemical activity of the plant photosynthetic apparatus, in particular in the range of low and medium light values.

Previously a low flavonoid content in the green leaves was revealed (3.0% dry mass), as well as a low total nitrogen content (1.8%), as compared to other plant species growing in Spitsbergen (Markovskaya and Shmakova 2012, 2017). From the literature it is known that flavonoids take part in the protection of plants from high light (Treutter 2006, Agati et al. 2013). However, *Stellaria humifusa* does not actively use this form of protection.

Previously, in the plants *Stellaria humifusa* growing in Spitsbergen, the antioxidant ferments activity was determined (Markovskaya et al. 2017). Data were obtained suggestive of a high catalase activity and a low peroxidase activity. The low peroxidase activity values, as well as the absence of any differences between its isoforms in *S. humifusa*, indicate that no excessive accumulation of hydrogen peroxide occurs, and the species is well-adapted to the environment. All the antioxidant load is taken by catalase, the activity level of which exceeds that of other herbaceous plants (Markovskaya et al. 2017), which can be associated with the involvement of this enzyme in oxygen metabolism (photorespiration) (Mhamdi et al. 2010). Previously it was shown that the exposure of the plant to stress factors leads to the accumulation of reactive oxygen intermediates, which results in an increase in catalase activity (Mhamdi et al. 2010). The increased light intensity along with a drop in the air temperature, when moving further towards higher latitudes and higher into the mountains, result in an increase in antioxidant content in plant leaves. These antioxidants help to remove radicals and serve as fermentative protection from photoinhibition. At the same time, researchers also note the essential role of catalase in the above-mentioned processes (Streb et al. 1997, Aguilera et al. 2002, Lütz 2010).

Apart from the increased catalase activity in the plants *Stellaria humifusa* growing in Spitsbergen, a high content of non-saturated lipids in the structure of the photosynthetic apparatus membrane system was observed, which contributes to its high functional activity (Markovskaya et al. 2017).

Thus, our findings, as well as the literature data, suggest that the protection of the photosynthetic apparatus from unfavorable environmental factors in *Stellaria humifusa* is achieved through both structural-functional mechanisms (the formation of a layer of dead leaves in the upper part of the mat and a high level of NPQ), and biochemical ones (the high level of catalase activity).

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