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THE INFLUENCE OF SOME ENVIRONMENTAL FACTORS ON CYTOLOGICAL AND BIOMETRIC PARAMETERS AND CHLOROPHYLL CONTENT OF *DESCHAMPSIA ANTARCTICA* DESV. IN THE MARITIME ANTARCTIC

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Under the environmental conditions of the Point Thomas Oasis (King George Island, the South Shetland Islands), we studied the influence of month-long artificial treatment with fresh water, salt water, and guano solution on the biometric characteristics, chlorophyll content, as well as the nuclear area of leaf parenchymal cells and nuclear DNA content, in a maritime Antarctic aboriginal plant Deschampsia antarctica. The modeled factors induced an increase in the generative shoot height and the length of the largest leaf, but did not influence the number of flowers. Treatment with guano caused an increase in the chlorophyll a and b contents, while fresh water treatment only led to some increase in chlorophyll a. Fluctuations of physiologically significant traits, such as the nuclear area and DNA content in the leaf parenchyma cells of D. antarctica, have been traced under the influence of the studied factors. Understanding of the hierarchy of influence of these factors as well as and sensitivity of plants of this species to external agents require further investigation.

Introduction. Antarctic is a region currently experiencing the most profound climate alteration on the planet. Its ice caps continue to melt and new areas are being released from ice as a consequence of warming in the maritime Antarctic [1, 2]. Under such circumstances, the advance and changes in the composition of plant communities may serve as a reliable indicator of the progress of these processes in different regions of the maritime Antarctic [3]. Among these communities, first and foremost attention should be paid to the formation of cenoses of the Antarctic herb tundra composed of two species of vascular plants: Antarctic hairgrass (*Deschampsia antarctica* Desv.) and Antarctic pearlworth (*Colobanthus quitensis* (Kunth.) Bartl.) that are probably most sensitive to warming, as well as of different mosses and macroalgae [4–6]. For using plant communities as indicators of climatic changes it is essential to study the variability of their composition, as well as the parameters of plant edificators in relation to the ecological conditions of their habitats. In this respect, the dependence of the composition of plant communities on the distance from guano sources is the most thoroughly studied topic so far [7–9]. Besides, a series of publications exists on the dependence of the Antarctic herb tundra formation on the basic natural gradients, such as the distance gradient from ocean coast to the brink of icecaps based, first of all, on differences in moisture [10, 11]. In these studies, most attention has been paid to the influence of ecological gradients on the composition of multispecies cenoses. The impact of the factors directly on certain species, namely the Antarctic herb tundra formation edificator *D. antarctica* remains poorly understood.

In this respect, there are only a few specific observations of the effects of direct influence of ecological factors on individuals of *D. antarctica in situ* which demonstrate a general growth rate increase in the Antarctic hairgrass in places where extra organic matter is available [12, 13]. Yet the mechanism of the reaction of an individual plant to changes in humidity or extra organics inflow may be realized in a variety of ways. The primary manifestation is in variability of the plant biometrics. Besides, several authors demonstrated a rapid chlorophyll content response to both internal and external factors [7, 14–16]. Under extreme conditions, polyploidization in separate tissues and in the whole plant organism has been reported [17–19]. In our previous research we also demon-

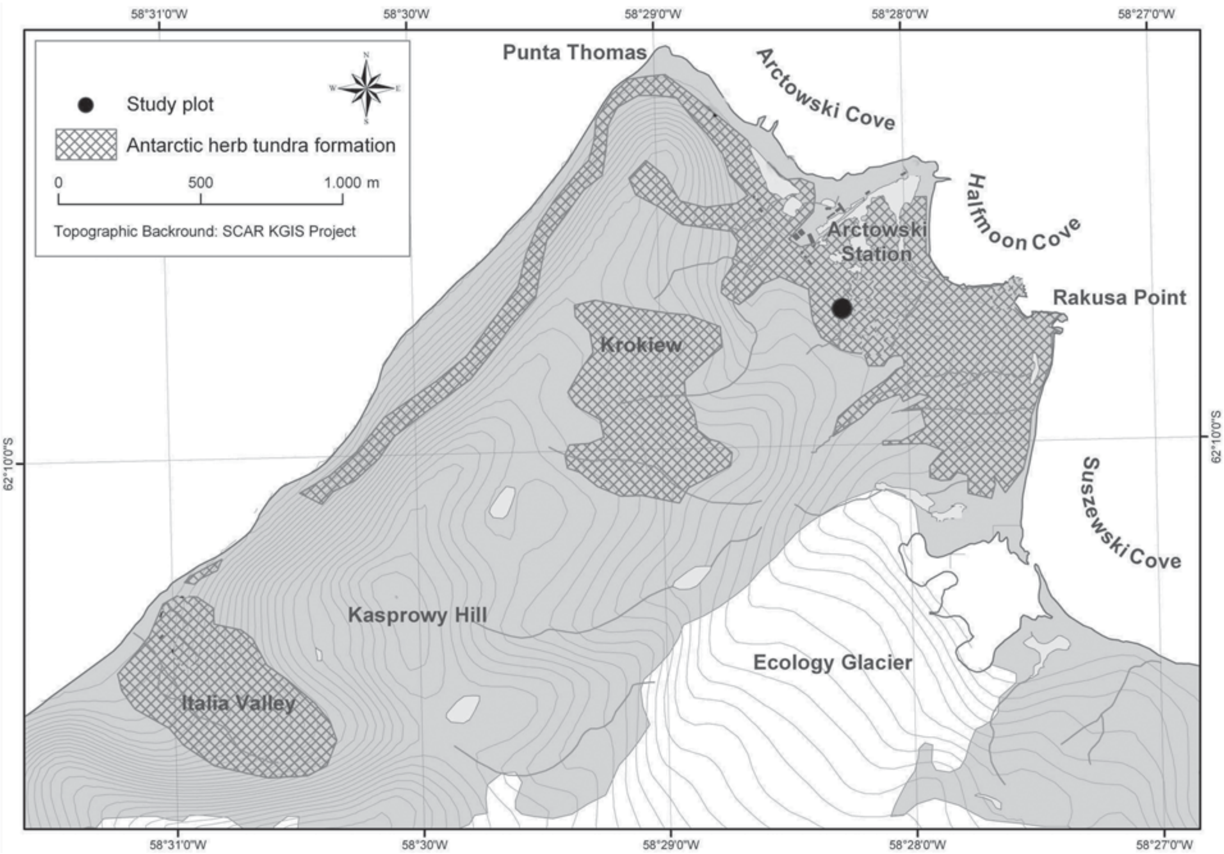

Fig. 1. Localization of study plot in the environs of Arctowski Station, King George Island, South Shetland Islands, maritime Antarctic

strated a reaction of nucleus square metrics and its DNA content to different growth conditions in *D. antarctica* growing in the Argentine Islands region [20, 21]. In view of this, the objective of the present research was to study the influence of modeled natural factors on some biometric, physiological, and cytogenetic parameters of *D. antarctica* plants *in situ*.

Materials and methods. *Study areas.* The experiment was conducted during the 30th Polish and the 10th Ukrainian expeditions (11.09.2005–02.09.2006) in the Point Thomas Oasis near the Polish Antarctic H. Arctowski Station on King George Island of the South Shetland Islands, maritime Antarctic. A plot was chosen with a relatively homogenous cover of *D. antarctica* (Fig. 1). Coordinates of the study area were determined using GPS (Garmin eTrex H).

Characteristics of the area with the studied plots are as follows: the Uplaz slopes region on the bank of an ice stream, S 62°09.735', W 58°28.253', 20 m above sea level, inclination 5–10°, 350 m away from the sea coast, mosaic inflow of guano from birds. Four 1 m^2 plots were designated within the chosen area.

Experimental setup. The first plot was irrigated with fresh water, plot 2 – with salt water, and plot 3 – with a guano solution. Plot 4 served as a control. The fresh water was taken from the nearby stream, the salt water originated from Admiralty Bay surface waters near the shore. The guano solution was prepared as follows: dry penguin guano was collected on the beach of Admiralty Bay and then drawn in a jar filled with fresh water in the proportion of approximately 100 g dry guano per 1 liter of water. Irrigation of all types was performed by pouring the solutions under *D. antarctica* clumps. Irrigation was performed on a daily basis at noon every day from 12/14/2005 until 01/12/2006.

Biometric analysis. At the beginning and in the end of the experiment, the following biometric parameters were measured at all plots: morphological features of generative plants, namely the height of the generative shoot (the distance between the ground surface and the top of the most distal spikelet in the inflorescence), the length of the largest leaf (from the ramification point to the leaf apex), the number of flowers in the inflorescence and the leaf condition (dry or green). For each plot, 20 individual plants were measured, the mean values calculated (SD allowed for) and compared.

Chlorophyll analysis. The influence of irrigation type on chlorophyll content in green leaves of *D. antarctica* was also addressed. This trait was studied on samples collected from all experimental plots on the 30th day of the experiment. For this purpose, we used the most common acetone method after McKinney (1941) modified by Starnes & Hadley (1965). The selected plant samples were grinded in a mortar with adding 85 % acetone solution. After homogenization, the samples were left in the mortar for 2–3 hours in a dark place for chlorophyll extraction from plant tissues. The resulting chlorophyll solution was filtered and poured into a graduated cylinder. The solution was brought up with acetone to the volume of 25 ml. Chlorophyll light absorbtion measurements were made using the spectrophotometer Cintra 20 (GBC Scientific Equipment, USA). Chlorophyll content in the leaves was calculated using the samples of McKinney and Wellburne [22, 23].

Cytological parameters. The following cytogenetic parameters of the leaves, parenchyma, and cells of *D. antarctica* from each plot were studied: the relative content of DNA (for which purpose the cells were stained after Feulgen) and the nucleus area. The leaves were fixed for the analysis on the 1st (12/14/2005), 7th (12/20/2005) and 30th (01/12/2006) day of experiment. For the DNA cytophotometry assay, the lowest third part of a leaf of a visibly undamaged mature generative plant was taken. After fixation in 100 % alcohol-acetic acid (3:1 v/v), material was kept in 70 % alcohol. Parenchyma of the leaves collected for the nucleus area and the relative DNA content (in follow nuclear DNA content) assays were then stained according to Feulgen [24] (Fig. 2).

The green light filter of the microscope optical system and the red Pal-n one of Asus V 3000 were used. Four samples from each locality were analyzed, every sample contained 25 nuclei. The analysis was performed using the digital camera Samsung CCD SAC-410 PA, videodriver Asus V 3000, and software packages Corel Draw 7.0, Photo Paint 7.0, and Scion Image (Scion Corporation, USA). The nucleus area on the images was measured in pixel units and then converted into SI. RDC was estimated by comparing the staining intensity of the nuclei to that of the anaphase nuclei of *D. antarctica* rootlet cells in which the quantity of DNA was estimated to be 4C [21]. The values obtained for both parameters were broken down into morphometric classes (Table 1).

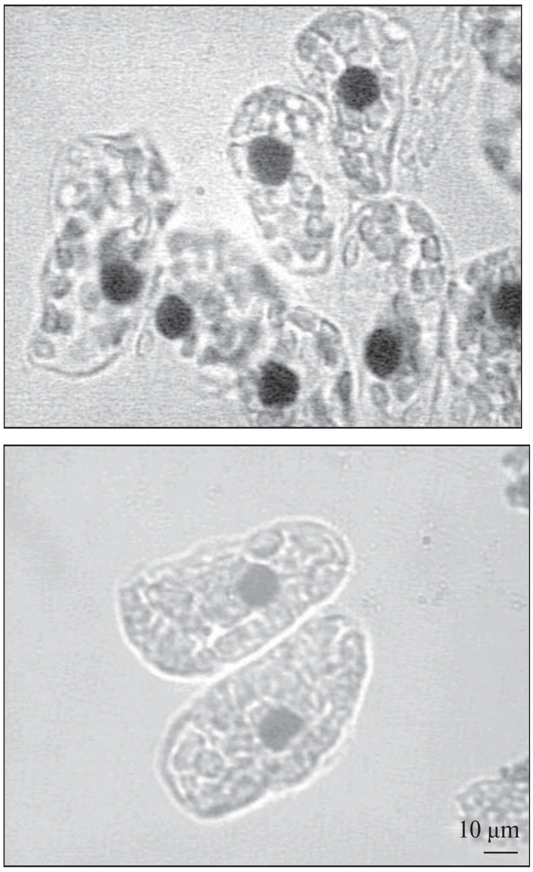

Fig. 2. The parenchyma leaf cells of *D. antarctica* stained for nuclear area and DNA content measure according to Feulgen

Based on the estimated frequency of each class, distribution curves were plotted for each parameter over all localities. To compare the curves and to determine the confidence intervals for correlation values we applied modificated the Median Test [25].

Table 1

The values of morphometric classes for nucleus area and the relative DNA content

Class number	Nucleus area, μm²	Nuclear DNA content, C
1	<10	<1
2	10–19.9	1–2.9
3	20–29.9	3–4.9
4	30–39.9	5–6.9
5	40–49.9	7–8.9
6	50–59.9	9–10.9
7	60–69.9	⩾11
8	⩾70	–

Results. The results of the experiment on the influence of some modeled natural factors on *D. antarctica* biometrics are summarized in Table 2.

The generative shoot height at fresh water and guano plots significantly exceeded that of the control plants. However, the largest difference was found at the plot watered with guano solution. Besides, both guano and the other two experimental factors induced significant increase of the length of the largest leaf, with the guano influence being the most profound. Under guano treatment conditions, the general *D. antarctica* growth enforcement was accompanied by appearance of new green leaves (unlike in plants from other plots, including control, with yellowish and partially dried leaves). The number of flowers per inflorescence never differed significantly from control, though in whole this trait may vary in different conditions [26].

As to chlorophyll *a*, its content increased after fresh water irrigation, but a particularly notable effect was observed under treatment with the guano solution. Chlorophyll *b* content significantly increased only in plants watered with the guano solution (Table 3).

During the whole experiment and under different treatments, we observed cells with the nuclear area within <10 – ⩾70 μm^2, while the relative nuclear DNA content ranged between <1–10.9 C. The range of variability, as well as the dominating class based on these traits in different experimental conditions, are summarized in Tables 4 and 5.

Experiments on the influence of some natural factors on the nucleus area and DNA content in the nuclei of leaf parenchyma cells of *D. antarctica* demonstrated that under control conditions statistically significant changes in nucleus area occurred on the 7th and 30th days of the experiment as compared to the original state (Table 6). Along with this, significant changes in DNA content in the nuclei of these cells in control plants have not been registered.

Salt water and guano treatments differed from control only in the absence of significant changes of the nuclear area on the 7th day of the experiment. Fresh water treatment didn't induce nuclear area changes on the 30th day (last day), as well as it did induce DNA content changes on the 30th day of the experiment unlike in all other types (in guano solution case this occurred on the 7th day), which renders them indistinguishable from control on 30th day.

Discussion. Comparisons between the influence of some natural factors on *D. antarctica* biometrics demonstrate that both at fresh water and guano plots the generative shoot height significantly exceeded that of control, probably due to certain additional watering.

The largest difference from the control was found at the plot watered with guano solution, probably due to a combination of two favorable factors: water as the main one and guano as reinforcement. In conditions where mineral complexes of the underlying rocks are rich [27], the main limiting factor seems to be humidity. Meanwhile none of the analyzed factors was found to induce significant changes in the number of flowers. This agrees quite well with data in literature, as several researchers reported seed yield in this species to increase under unfavorable conditions, such as clump densening, decrease water or biogens availability. In relatively favorable conditions seed yields decreased [13, 28]. However, during our experiment, irrigation, and, thus, better conditions, did not produce any decrease in the number of flowers per inflorescence. This allows speculation that this trait may be highly conservative. Another explanation could be that the experimental treatment period might have been insufficient for significant changes to occur. However, it should be noted that treatment of each type was performed during the key vegetation period for *D. antarctica* (30 days), when mean temperatures remain above 0 °C. Additionally, during the whole experimental period a general increase in the growth rate of hairgrass was noticed as indicated by the

Table 2

The influence of some modeled natural factors on biometrics of the generative plants of *D. antarctica*

Variant	Generative shoot height, cm	Length of biggest leaf, cm	Number of flowers	Leaf condition
Control	2.7 ± 0.19/0.7	0.9 ± 0.07/0.1	8.2 ± 0.35/2.4	Some dry leafs
Fresh water	3.2 ± 0.18/3.2	1.5 ± 0.09/1.8	9.0 ± 0.64/8.3	Some dry leafs
Sea water	3 ± 0.21/0.9	1.2 ± 0.08/0.1	8.1 ± 0.42/3.6	Some dry leafs
Guano solution	3.8 ± 0.18/0.68	2.2 ± 0.13/0.3	7.8 ± 0.56/6.3	Without dry leafs

appearance of green leaves at the plot with guano treatment.

Only after our experiment had ended we found out that Polish researchers conducted a similar experiment during the season of 1988 near the Arctowski Station. They planted clumps of similar size of *D. antarctica* та *Colobanthus quitensis* (in three combinations and three trials) which were irrigated with a guano solution, urine, and fresh water. However, no indications on how long the treatment continued and how regular it was were present in publications. In 1990, it was revealed that *C. quitensis* died out completely, while hairgrass grew slowly, and its cover had closed in. The best growth was observed at plots fertilized with a urea solution, guano-treated plants felt a little worse, and only a few plants vegetated at fresh water irrigated plots [12, 13]. The general growth increase under increased nutrient content conditions is known for other plants as well [29].

The increase of the positive effect of watering and solved nutrients is corroborated by the elevated levels of chlorophyll *a* content under both treatment types, while their complementary action only in the case with guano solution treatment. Along with this, it is known that both increase in available moisture and the effect of organics may, depending on conditions, increase, decrease, or leave without changes chlorophyll content [29–31].

It can be noticed also that the registered difference in chlorophyll *a* and *b* contents in control possibly explains the yellowish color of the aboveground parts of hairgrass from the control plot. In the same time, the rich green color acquired by the plants at the guano treated plot was accompanied with a significant increase in chlorophyll *b* content. Chlorosis resulting from misbalance in the ratio between chlorophylls *a* and *b* is known from literature [32]. It has also been pointed out that

Table 3

The influence of the some modeled natural factors on the content of leaf chlorophyll *a* and *b* (mg g^{-1} DW) in *D. antarctica in situ*

Variant	Chlorophyll		
	a	*b*	*a + b*
Control	1,286 ± 0,149	0,693 ± 0,286	1,979 ± 0,403
Fresh water	2,107 ± 0,206	0,685 ± 0,129	2,800 ± 0,322
Sea water	1,259 ± 0,262	0,701 ± 0,321	1,960 ± 0,581
Guano solution	3,234 ± 0,178	1,304 ± 0,100	4,539 ± 0,224

increase in chlorophyll *b* content may take place due to considerable ramification of a plant and, thus, decreased availability of light to some parts of the plant [29], which has not been registered in our case.

Increase in chlorophyll *a* and *b* contents as a reaction to favorable conditions, specifically increased availability of water, is probably not restricted to Antarctic vascular plants; as such mechanism has also been demonstrated for plants, for instance for maritime Antarctic mosses [7].

In case of cytological traits, it should be taken into account that the revealed changes of the nuclear area in control during the whole experimental period indicate that all four plots experienced equal impact of natural fluctuations of the available moisture and other factors. Meanwhile the difference in the effect of all studied factors from that in control allows considering these factors as primary causative agents of changes in the nuclear area and DNA content, as well as of, probably, cell activity in all three cases. Similarity has been found between salt water and guano treatment effects which both do not induce nuclear area changes on the 7th day. This trend in both indicated cases disappears by the end of the experi-

Table 4

The influence of the some modeled natural factors on the general range of variability (*a*) and the dominant class (*b*) and its percentage frequency based on the nuclear area of leaf parenchyma cells in *D. antarctica*

Variant	1st day		7th day		30th day	
	a	*b*	*a*	*b*	*a*	*b*
	μm²					
Control	20–59.9	30–30.9 (63 %)	20–69.9	30–30.9 (49 %)	20 – 79.9	40–49.9 (36 %)
Fresh water	20–59.9	30–30.9 (58 %)	20–69.9	30–30.9 (46 %)	20 – ⩾70	50–59.9 (37 %)
Sea water	10–59.9	30–30.9 (52 %)	20–59.9	30–30.9 (69 %)	<10 – ⩾70	40–49.9 (32 %)
Guano solution	20–69.9	40–40.9 (42 %)	20–69.9	30–39.9 (42 %)	20 – ⩾70	40–49.9 (27 %)

Table 5

The influence of the some modeled natural factors on the general variability range (*a*) and the dominant class (*b*) based on the relative nuclear DNA content in leaf parenchyma cells of *D. antarctica*

Variant	1st day		7th day		30th day	
	a	*b*	*a*	*b*	*a*	*b*
	C					
Control	1–8.9	3–4.9 (72 %)	1–6.9	3–4.9 (85 %)	1–12.9	5–6.9 (49 %)
Fresh water	1–6.9	3–4.9 (82 %)	1–6.9	3–4.9 (80 %)	1–8.9	3–4.9 (72 %)
Sea water	3–8.9	3–4.9 (65 %)	1–6.9	3–4.9 (80 %)	1–10.9	3–4.9 (53 %)
Guano solution	1–8.9	3–4.9 (72 %)	1–6.9	3–4.9 (75 %)	<1–10.9	5–6.9 (55 %)

Table 6

The influence of the some modeled natural factors on changes in the nuclear area and DNA content of leaf parenchyma cells of *D. antarctica in situ*

Variant	7st day		30st day	
	Nuclear area	DNA content	Nuclear area	DNA content
Control	$\chi^2 > 3.84$*	–	$\chi^2 > 3.84$	–
Fresh water	$\chi^2 > 3.84$	–	–	$\chi^2 > 3.84$
Sea water	–	–	$\chi^2 > 3.84$	–
Guano solution	–	$\chi^2 > 3.84$	$\chi^2 > 3.84$	–

* Events of significant change of a parameter on the day 7 and day 30 from the start of the experiment under different treatment types calculated by median test with given probability of $\chi^2 > 3.84$ have been presented.

ment, which may be connected with fluctuations in development of physiological reactions in plants under the treatment conditions.

The statistically significant impacts of guano treatment on the DNA content registered on the 7th (in guano solution case) and 30th (in fresh water case) days seem to indicate that fluctuations of physiological processes (which are detected by this characteristic) took place during the whole period or at some stages of the experiment.

Therefore, the influence of such factors like irrigation with fresh water, salt water or guano solution directly induces a reaction of physiologically important traits in individual plants of *D. antarctica*, which may cause changes in their biomass, reproductive potential, and cenotic activity, and consequently potential variability of the cenoses. Fluctuations of physiologically significant traits, such as the nuclear area and DNA content in the leaf parenchyma cells of *D. antarctica*, under the influence of the studied factors have been traced. Understanding the hierarchy of the influence of these factors, as well as the sensitivity of the plants of this species to external agents, requires further investigation.

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ВЛИЯНИЕ НЕКОТОРЫХ ФАКТОРОВ ОКРУЖАЮЩЕЙ СРЕДЫ НА ЦИТОЛОГИЧЕСКИЕ И БИОМЕТРИЧЕСКИЕ ПАРАМЕТРЫ, А ТАКЖЕ СОДЕРЖАНИЕ ХЛОРОФИЛЛА *У DESCHAMPSIA ANTARCTICA* DESV. ПРИБРЕЖНОЙ АНТАРКТИКИ

В условиях оазиса Поинт Томаса (остров Короля Георга, Южные Шетлендские острова) было изучено влияние искусственной обработки в течение месяца пресной, морской водой и раствором гуано на биометрические показатели, содержание хлорофиллов, а также площадь ядра паренхимных клеток листка и содержание ядерной ДНК в них у растений аборигенного вида Прибрежной Антарктики – *antarctica*. Искусственное влияние факторов окружающей среды вызвало увеличение высоты генеративного побега и длины наибольшего листа, но не влияло на количество цветков. Обработка раствором гуано увеличивала содержание хлорофиллов *a* и *b*, а пресной водой – только содержание хлорофилла *a*. Выявлены признаки флуктуации физиологически значимых параметров: площади ядра и содержания ДНК клеток паренхимы листа *D. antarctica*, для выяснения иерархии которых, а также степени чувствительности к внешним факторам требуются более детальные исследования.

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ВПЛИВ ДЕЯКИХ ФАКТОРІВ ДОВКІЛЛЯ НА ЦИТОЛОГІЧНІ ТА БІОМЕТРИЧНІ ПАРАМЕТРИ, А ТАКОЖ ВМІСТ ХЛОРОФІЛУ *DESCHAMPSIA ANTARCTICA* DESV. ПРИБЕРЕЖНОЇ АНТАРКТИКИ

В умовах оази Поінт Томаса (острів Короля Георга, Південні Шетлендські острови) було вивчено вплив штучної обробки протягом місяця прісною, морською водою та розчином гуано на біометричні показники, вміст хлорофілів, а також площу ядра паренхімних клітин листка та вміст ядерної ДНК в них, у рослин аборигенного виду Прибережної Антарктики – *antarctica*. Штучний вплив факторів довкілля викликав збільшення висоти генеративного пагону та довжини найбільшого листка, але не впливав на кількість квіток. Обробка розчином гуано збільшувала вміст хлорофілів *a* та *b*, а прісною водою – тільки вміст хлорофілу *a*. Виявлено ознаки флуктуації фізіологічно-значущих параметрів: площі ядра та вмісту ДНК клітин паренхіми листка *D. antarctica*, з'ясування ієрархії яких, а також ступеню чутливості до зовнішніх факторів потребує детальнішого дослідження.

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