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ANATOMICAL AND FUNCTIONAL FEATURES OF *DESCHAMPSIA ANTARCTICA* (POACEAE) LEAF BLADE GROWING ON THE ARGENTINE ISLANDS

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Abstract. The **objective** of the work was to perform the comparative studies of the anatomical structure and reactive oxygen species (ROS) content in leaves of *Deschampsia antarctica* plants growing in extreme climatic conditions of Antarctica (Skua and Galindez Islands). The leaf anatomy and surface ultrastructure were investigated by the **methods** of light and scanning electron microscopy. To determine the localization of monolignines a cytochemical method of dyeing tissues was used. The ROS content was registered by measuring the spontaneous chemiluminescence (SCL). The obtained **results** showed the similarity of leaf anatomical and ultrastructural features in plants collected on Scua and Galindez Islands. The localization of two monolignines (syringyl and quaiacyl) detected in leaf cell walls was also similar in the investigated plants. Syringyl is mainly localized in the walls of epidermal cell and quaiacyl is mainly localized in the walls of mesophyll cells and vessels. In epidermal cell walls, the syringyl relative content exceeded the quaiacyl content 6-8 times. The SCL level in *D. antarctica* leaves is corresponded to mean values of leaf luminescence in other species under the normal conditions. This may indicate the adaptation of species to harsh habitats. In our opinion, it should be paid special attention on the study of *D. antarctica* cell metabolism and its regulation for better understanding the mechanisms of its survival in the conditions of the Maritime Antarctic.

Key words: *Deschampsia antarctica*, anatomical structure, ultrastructure, lipid peroxidation, lignin, adaptation.

АНАТОМІЧНА І ФУНКЦІОНАЛЬНА ХАРАКТЕРИСТИКА ЛИСТКОВОЇ ПЛАСТИНКИ *DESCHAMPSIA ANTARCTICA* (POACEAE), ЩО РОСТЕ НА АРГЕНТИНСЬКИХ ОСТРОВАХ

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Реферат. Метою роботи було проведення порівняльних досліджень анатомічної структури та вмісту активних форм кисню (АФК) в листках *Deschampsia antarctica* (Poaceae), що росте в екстремальних кліматичних умовах Антарктики (острови Скуа і Галіндез). Для досліджень анатомічної структури та ультраструктури поверхні листків використовували **методи** світлової та скануючої електронної мікроскопії. Локалізацію монолігнінів визначали за допомогою цитохімічного методу фарбування тканин, вміст АФК в листках – методом реєстрації спонтанної хемілюмінесценції (СХЛ). **Результати** засвідчують ідентичність анатомічної структури та ультраструктури поверхні листків у рослин, зібраних в різних місцезростаннях. Локалізація в листках двох монолігнінів (сирингіла та гваяцила) в клітинних стінках листків досліджених рослин була також подібною. Встановлено, що сирингіл, в основному локалізований в клітинних стінках епідермісу, підтримуючи їхню механічну міцність, а гваяцил, що забезпечує гнучкість стінок, локалізований в основному в стінках клітин мезофілу і судин. Вміст сирингілу в стінках клітин епідермісу перевищував рівень гваяцилу у 6–8 разів. Рівень СХЛ в листках *D. antarctica* дорівнював середньому значенню люмінесценції листків інших видів в нормальних умовах. Можна зробити висновки, що отримані результати можуть бути показниками адаптації цього виду до екстремальних умов. На нашу думку, посилення уваги до вивчення клітинного метаболізму та його регуляції у рослин *D. antarctica* сприятиме подальшому пізнанню механізмів їхнього виживання в умовах Прибережної Антарктики.

Ключові слова: *Deschampsia antarctic*а, анатомічна структура, ультраструктура, перекисне окислення, лігнін, адаптація.

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АНАТОМИЧЕСКАЯ И ФУНКЦИОНАЛЬНАЯ ХАРАКТЕРИСТИКА ЛИСТОВОЙ ПЛАСТИНКИ *DESCHAMPSIA ANTARCTICA* (POACEAE), ПРОИЗРАСТАЮЩЕЙ НА АРГЕНТИНСКИХ ОСТРОВАХ

Е. Л. Кордюм[1], Е. М. Недуха [1], Ю. В. Овчаренко [1], С. И. Жадько[1], Г. Ф. Иваненко [1], В. В. Лоя [2]

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Реферат. Целью работы было проведение сравнительных исследований анатомической структуры и содержания активных форм кислорода (АФК) в листьях растений *Deschampsia antarctica* (Poaceae), произрастающей в экстремальных климатических условиях Антарктики (острова Скуа и Галиндез). Для исследований анатомии и ультраструктуры поверхности листьев использовали **методы** световой и сканирующей электронной микроскопии. Локализацию монолигнинов определяли с помощью цитохимического метода окрашивания тканей, содержание АФК в листьях определяли методом регистрации спонтанной хемилюминесценции (СХЛ). **Результаты** подтверждают идентичность анатомической структуры и ультраструктуры поверхности листьев у растений, собранных в различных местах их произрастания. Локализация двух монолигнинов (сирингила и гваяцила) в клеточных стенках листьев исследованных растений также была подобной. Установлено, что сирингил в основном локализован в клеточных стенках эпидермиса, поддерживая их механическую прочность, а гваяцил, обеспечивающий гибкость стенок, локализован в основном в стенках клеток мезофилла и сосудов. Содержание сирингила в стенках эпидермальных клеток превышало содержание гваяцила в 6–8 раз. Уровень СХЛ в листьях *D. antarctica* равнялся среднему значению люминесценции листьев других растений, растущих в нормальных условиях. Полученные результаты позволяют сделать выводы об адаптации растений *D. antarctica* к экстремальным условиям. По нашему мнению, дальнейшие исследования клеточного метаболизма и его регуляции у растений *D. antarctica* углубят представления о механизмах их выживания в Прибрежной Антарктике.

Ключевые слова: *Deschampsia antarctica*, анатомическое строение, ультраструктура, перекисное окисление, лигнин, адаптация.

1. Introduction

Deschampsia antarctica (Poaceae) is the only natural grass species growing in the Antarctic geobotanical zone (Gielwanowska, Szczuka, 2005). The existence of *D. antarctica* plants in the severe climatic conditions is possible owing to certain mechanisms for growth and survival. The studies of leaf anatomy, its surface ultrastructure, and functioanal activity may help to understand the mechanisms of adaptation of *D. antarctica* plants to the extreme environment. At the normal conditions reactive oxygen species (superoxide anion radical, hydrogen peroxide) are permanently generated in cells and may cause oxidative-destructive effects in plant cells and perform signalling functions also. The ROS content sharply increases under different stresses creating a threat of oxidative destruction (Mittler et al., 2004). The activity of antioxidant enzymes, in particular superoxide dismutase, ascorbate peroxidase, catalase, peroxy redoksin, enchances in response to ROS stressed increasing, that to a large degree determines plant stability and adaptation (Santos, Rey, 2006; Kolupaev, Karpets, 2014). The peculiarities of the leaf mesophyll ultrastructure of *D. antarctica* (Gielwanowska et al., 2005; Szczuka et al., 2013) clearly demonstrate the high level of metabolism and production of ATP. ATP is an universal source of energy in cells of all live organisms. Earlier it was reported that levels of sucrose and fructans in leaves and roots of *D. antarctica* were higher in comparison with other cereals (Zuciga et al., 1996). The maximum accumulation of these substances occurred by the end of summer. It was also found the correlation of an activity of sucrose-phosphate-synthase (SPS) with day duration length and low temperature (Zunica-Feest et al., 2003). The highest SPS activity and the highest sucrose content in leaves were determined in cold-acclimated plants under long day. On authors' opinion such unusually high accumulation of sucrose and fructose, as one of the protective mechanisms against low temperature, and capacity to rapid growth during the short vegetation period has allowed *D. antarctica* to adjust to the conditions of the Maritime Antarctic.

2. Materials and methods of research

The specimens of *D. antarctica* were collected at the Galindez and Scua Islands in March 2016 during the 20th Ukrainian Antarctic expedition (Fig.1).

The content of ROS in leaves of *D. antarctica* plants was determined by the method of registration of spontaneous luminescence (SCL) with a chemiluminometer CLMC-01 (Ukraine). The intensity of SCL was measured in impulses per second per gram of fresh weight (imp/sec/g). Native leaves were placed in the cuvette of a device, and luminescence was measured in real time (Jadko, 2012).

Fig. 1. Scheme of the sites where samples of *Deschampsia antarctica* (Poaceae) plants were collected (Galindez Island and Scua Island).

To study the ultrastructure of the leaf surface, segments middle part of leaf blade were fixed in 2% paraformaldehyde in phosphate buffer, dehydrated in the ethanol series and acetone. The samples were mounted on tables by the standard technique, sputtered with gold, and examined with a JSM-6060 scanning electron microscope (JEOL, Japan). Leaf anatomy was studied by using segments of the leaf blade with a size of 0,5 X 1,0 cm. Leaf segments were fixed by 2,5 % glutaraldehyde and 1 % OsO4, dehydrated in the ethanol series and acetone, and placed in the mixture of epoxy resins (epon–araldite) on the generally accepted method. The cross sections (0,5-1,0 µm) were obtained on an ultramicrotome RMC MTXL (USA), stained with 1 % methylene blue and 0,12 % toluidine blue, and examined with light microscopes NF and Axioscope (Carl Zeiss, Germany).To determine the distribution and localization of monolignins used the cytochemical method with staining the samples by solution of 0,1% 2-aminoethyl ether-biphenyl carboxylic acid. Stained samples of leaf blades were examined with a laser scanning microscope LSM 5 Pascal (Carl Zeiss, Germany) under an excitation wave length of 380 nm and the absorption wave length 430 nm for quaiacyl; and under excitation wave length of 490 nm and the absorption wave length of 520 nm for syringyl.

3. Results and Discussion

ROS are permanently generated in cells and are under control of the antioxidant system (Mittler et al., 2004; Dietz, 2008). The main compartments for ROS production in plant leaf photosynthesizing cells are mitochondria, chloroplasts and peroxysomes (Foyer, Noctor, 2003).

ROS signals are generated in different sites. In different metabolic reactions ROS signals may be combined to produce a new signal, as well as one signal can play a dominant role showing an epistatic effect (Moller, Sweetlove, 2010). ROS also initiate post-translational modification of proteins, especially by the oxidation of sulfhydryl groups that leads to the changes in their structure and functions. Free radicals attack also membrane lipids containing carbon-carbon double bonds, especially polyunsaturated fatty acids. This process is generally known as a process of lipid peroxidation. The importance of the membrane lipid physical state is evidenced by the fact that lipids may control the physiological state of a membrane organelle by modifying its biophysical aspects, such as the polarity and permeability. Lipids also have a key role in biology as signaling molecules (Ayala et al., 2014). It is of interest to study a trigger role of ROS in the mechanism of epigenetic regulation of gene expression by the changes in histone acetylation (Jadko, 2015). It should be noted that ROS content is well determined by the registration of spontaneous chemiluminescence (SCL) from live cells, as the increased content of malondialdehyde. The malondialdehyde is one of final products of lipid peroxidation and it is revealed only at the more late stages of stress action (Jadko, 2012). The SCL method may be successfully used to study a role of ROS as secondary messengers in the induction/activation of plant stress-reactions and adaptation to the influence of environmental unfavourable factors. The intensity of SCL in leaves of plants from the islands Scua and Galindez was 36±4 and 43±4,5 imp/sec/g in the average, respectively (Fig. 2). The level of SCL in *D.* a*ntarctica* leave is corresponded to mean values of leaf luminescence in the normal conditions, for example, it was 28-33 imp/sec/g in *Arabidopsis thaliana*, (Jadko, 2012) and 27- 38 imp/sec/g in *Pisum sativum, Zea mays*, and *Triticum aestivum* (Tarusov, Veselovskii,1978). Therefore, the obtained data may be evidence of *D. antarctica* adaptation to the conditions of its habitat.

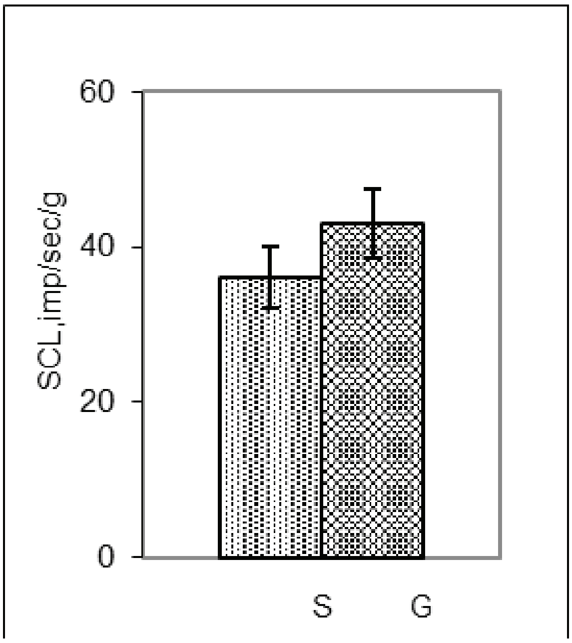
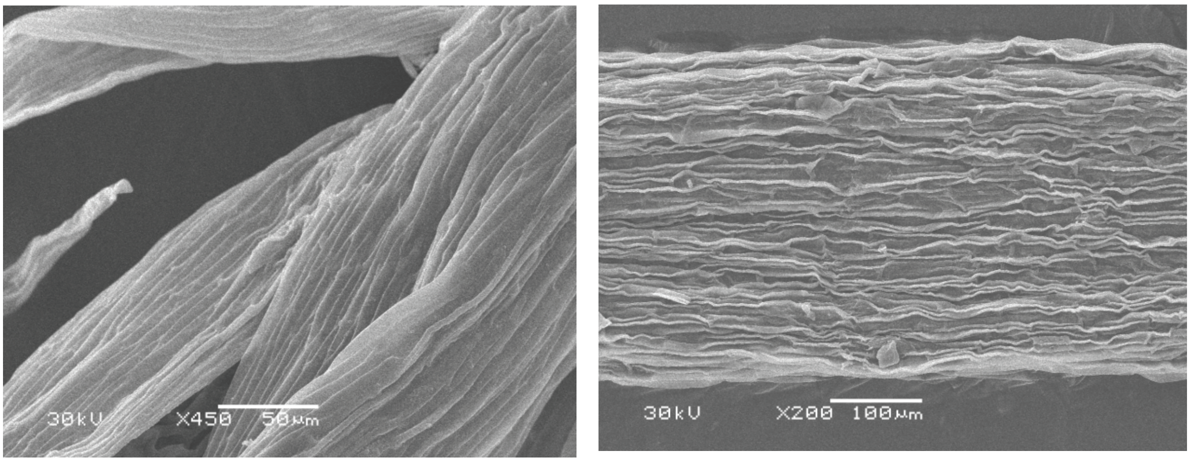


Fig. 2. The SCL intensity in leaves of *Deschampsia antarctica* (Poaceae) plants from the islands Scua (S) and Galindez (G).

The leaf surface ultrastructure in plants collected on Scua Island and Galindez Island is identical. The adaxial and abaxial surfaces of a leaf blade were covered with high cuticular ribs which situated parallel to the longitudinal axis of a leaf blade. Cuticular ribs on the leaf abaxial surface are almost straight (Fig. 3a). Cuticular ribs on the leaf adaxial surface were slighty wavy-grained (Fig. 3b). A height of ribs varied from 10 μm to 12 μm, a breadth varies from 4 μm to 6 μm.



a b

Fig. 3. The structure of leaf abaxial (a) and adaxial (b) surface in *Deschampsia antarctica* (Poaceae).

The similarity of morphological and anatomical traits of leaves in *D. antarctica* plants collected on the islands Scua and Galindez was shown (Fig.4 and 5). Oval cells of the adaxial epidermis were covered with the cuticle layer.

Dumbbell stomata were situated at the epidermis level. The density of stomata was higher at the leaf abaxial surface than at the adaxial ones. Epidermal cells adjoined to guard cells were situated parallel to the leaf longitudinal axis and do not differ from the constitutive ones. Undifferentiated mesophyll consisted of thin-wall, isodiametrical and loosely located cells. A characteristic feature of all investigated samples was the presence of large gas spaces in the mesophyll (aerenchyma). Chloroplasts were situated on the periphery of mesophyll cells.

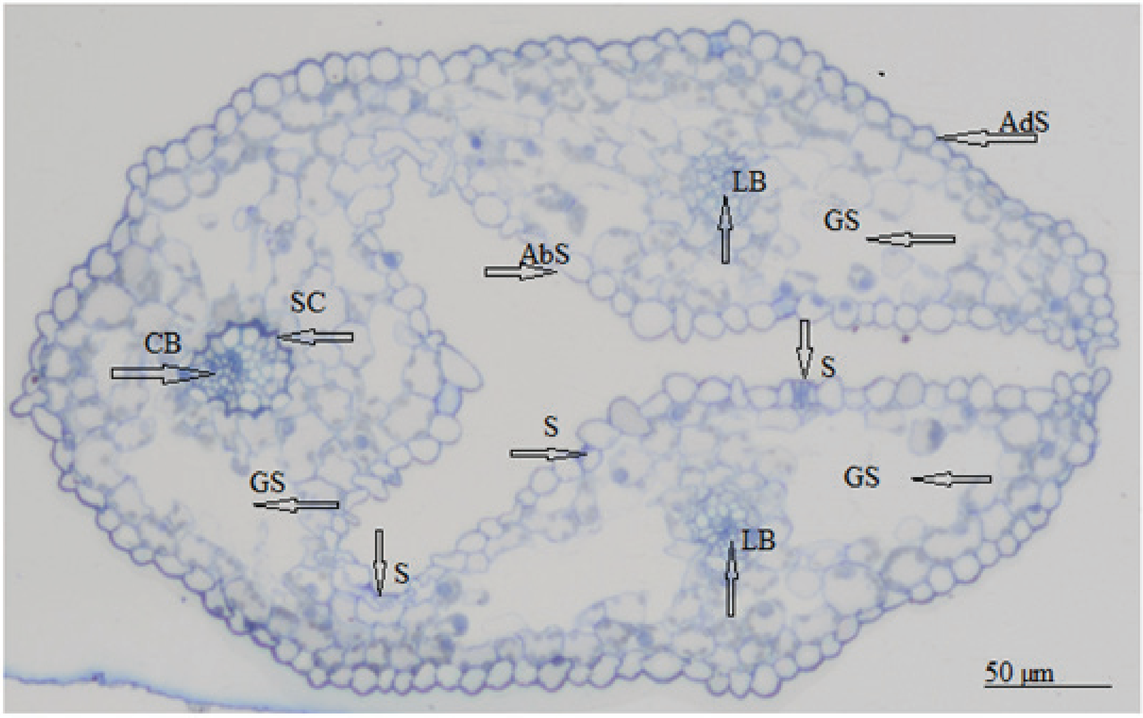


Fig. 4. Cross section of a leaf blade of *Deschampsia antarctica* (Poaceae) from Scua Island. Abbreviations: AdS –adaxial surface, AbS –abaxial surface, CB –central bundle, LB –lateral bundle, S –stomate, GS – gas space, SC–sheath cell.

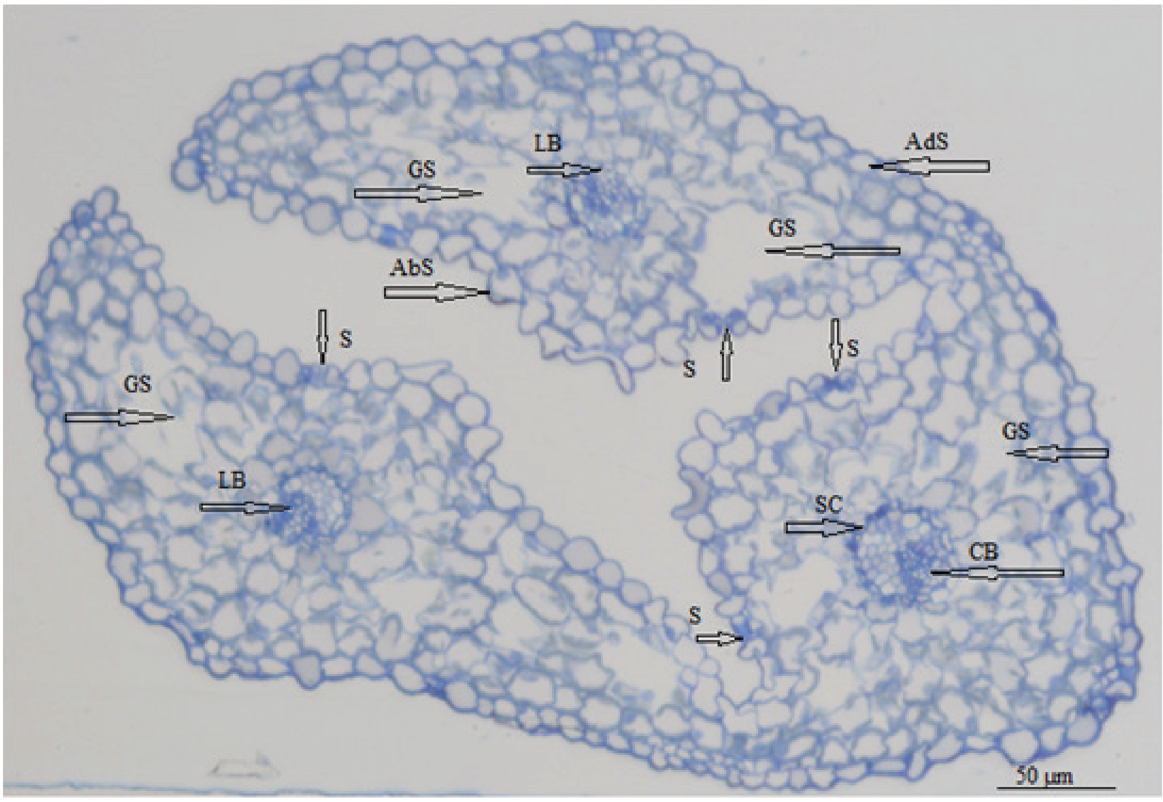
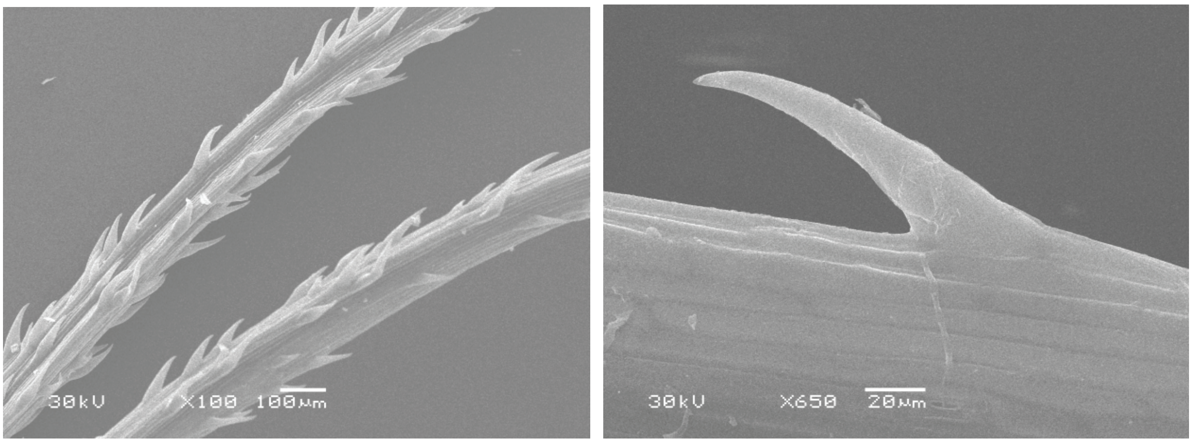


Fig. 5. Cross section of a leaf blade of *Deschampsia antarctica* (Poaceae) from Galindez Island

(abbreviations are the same as on the Fig. 4).

The leaf conducting system consisted of three closed vascular bundles: one central and two lateral, containing the xylem and phloem elements. Bundles were surrounded by tightly located sheath cells. The sheath cells separated them from mesophyll cells. Collenchyme fibers adjoining to the epidermis give the mechanical strength to a leaf. Folding leaf blade may protect stomata in the conditions of low temperature and elevated humidity, diminishes a rate of transpiration and supports gas exchange.

It was shown that branches of the panicle were downy. They were covered with simple unbranched trichomes (Fig. 6). An average length of trichome was 100 µm, an average breadth in its basis was 40 µm, the distance between trichomes was 80 – 200 µm and an average density per 1 mm 2 was ≈ 72.



a b

Fig. 6. Downy branches of the panicle of *Deschampsia antarctica* (Poaceae): a – general view, b – trichome.

The presence of two monolignins (syringyl and quaiacyl) were revealed in leaf cell walls using the cytochemical methods and confocal microscopy. It was established that syringyl is mainly located in epidermal cell walls supporting their mechanical strength, and quaiacyl is mainly located in walls of mesophyll cells and vessels in conducting bundles providing flexibility of walls (Fig. 7). The relative content of syringyl in epidermal cell walls exceeds the relative content of quaiacyl in 6-8 times.

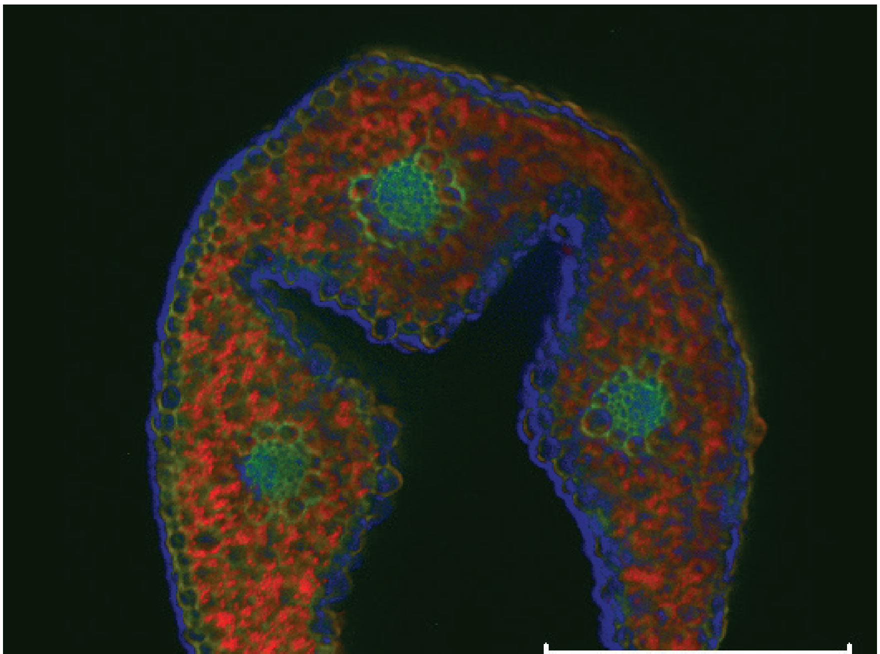


Fig. 7. Fluorescence of lignin in leaf cell walls of *Deschampsia antarctica* (Poaceae) after staining with 0.1% 2-aminoethyl ether-biphenyl carboxylic acid (syringyl fluoresces with blue color, quaiacyl – with green color, red color –autofluorescence of chlorophyll).

4. Conclusions

The morphological and anatomical traits of *D. antarctica* plants leaves investigated by us and collected on the islands Scua and Galindez are similar to plants leaves characteristics of the species from the other regions of Maritime Antarctic (Romero et al., 1999; Gielwanowska, Szczuka, Bednara et al., 2005 Szczuka et al., 2013). The high relative content of monolignin syringyl in epidermal cell walls, especially of the abaxial epidermis was determined for the first time. It jointly with cuticular ribs provides the mechanical strength of leaves, as well as the protection from surplus penetration of water. The normal level of SCL in leaves of investigated plants may indicate their adaptation to the environment. The literature data reported the genetic variability and phenotypic plasticity of *D. antarctica* populations (Romero et al., 1999; Gielwanowska, Szczuka, 2005; Gielwanowska et al., 2005; Chwedorzewska et al., 2008; Szczuka et al., 2013; Amosova et al., 2015), while a search of any obvious unique or specialised adaptations in this species to the harsh conditions of the Maritime Antarctic did not give positive results. In addition, the first performed investigations of allelopatic properties of the soil samples collected under mosses and under mosses with *D. antarctica* plants on the islands Scua and Galindez, showed the favorable conditions for plant growth. The obtained results may be considered as a good example of coexistance of *D. antarctica* plants and mosses for survival in the harsh habitats. Therefore, in our opinion, a special attention should be paid to the study of cell metabolism and its regulation, functioning of energetic organelles – chloroplasts and mitochondria and their interaction, and epigenetic regulation in gene expression also for deeper understanding the structural and functional organization of *D. antarctica* plants providing their growth and fruiting in the conditions of the Maritime Antarctic.

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6. References

1. Amosova, A. V., Bolsheva, N. L., Samatadze, T. E., Twardovska, M. O., Svyatoslav, A., Zoshchuk, I.O., Badaeva, E. D., Kunakh, V. A., Muravenko, O.V. 2015. Molecular cytogenetic analysis of *Deschampsia antarctica Desv.* (Poaceae), Maritime Antarctic. PLOS. http://dx.doi.org/10.1371/journal.pone.0138878
2. Ayala, A., Mucoz, M.F., Argüelles, S. 2014. Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. *Oxidative Medicine and Cellular Longevity.* ID 360438. http://dx.doi.org/10.1155/2014/360438
3. Chwedorzewska, K. J., Gielwanowska, I., Szczuka, E., Bochenek, A. 2008. High anatomical and low genetic di-versity in *Deschampsia antarctica Desv.* from King George Island, the Antarctic. Pol. Polar Res., 29, 377–386.
4. Dietz, K.-J. 2008. Redox signal integration: from stimulus to networks and genes. *Physiol. Plant*.,133, 459–468.
5. Foyer, C. H., Noctor, N. 2003. Redox sensing and signalling associated with reactive oxygen in chloroplasts, peroxisomes and mitochondria. *Physiol. Plant*.,119, 355–364.
6. Gielwanowska, I., Szczuka, E. 2005. New ultrastructural features of organelles in leaf cells of *Deschampsia antarctica* Desv. *Polar Biol.*, 28, 951–955.
7. Gielwanowska, I., Szczuka, E., Bednara, J., Gorecki, R. 2005. Anatomical features and ultrastructure of *Deschampsia antarctica* (Poaceae) leaves from different growing habitats. *Ann. Bot.*, 96, 1109–1119.
8. Jadko, S.I. 2012. Ranneye uvelicheniye soderzhaniya aktivnykh form kisloroda I aktivnosti askorbat peroksi-dazy I katalazy v list'yakh Arabidopsis thalianapri osmoticheskom i oksidativnom stessakh [Early increasing the content of reactive oxygen species and the activity of ascorbate peroxidase and catalase in leaves of Arabidopsis thalianaplants under osmotic and oxidative stresses]. *Visnyk Kharkiv. natsionalnogo agrarnogo universytetu. Seriya Bioilogiya*.[The Bulletin of Kharkiv National Agrarian University. Ser. Biology]. 3(27), 58–64.
9. Jadko, S. I. 2015. Histone deacetylase activity and reactive oxygen species content in the tissue culture of Arabi-dopsis thalianaunder normal conditions and development of acute osmotic stress. *Ukr. Biochem. J.*, 87, 57 – 62.
10. Kolupaev, Iu.E., Karpets ,Iu.V. 2014. Aktyvnye formy kisloroda i stressovyi syhnalynh u rastenyi [Reactive oxygen species and stress signaling in plants]. *Ukr. Biochem. J.*, 86 (4), 18–35.
11. Mittler R., Vanderauwera S., Gollery M., Breusegem F.V. 2004. Reactive oxygen gene network of plants. *Trends Plant Sci.,* 9, 490–498.
12. Moller, I. M., Sweetlove, L. J. 2010. ROS signaling-specificity is required. *Trends Plant Sci*., 15, 370–374.
13. Romeo, M., Casanova, A., Iturra, G., Reyes ,A., Montenegro, G., Alberdi, M. 1999. Leaf anatomy of *Deschampsia antarctica (Poaceae)* from the Maritime Antarctic and its plastic response to changes in the growth conditions. *Revista Chilena de Historia Natural*, 72, 411–425.
14. Santos, C.V., Rey, P. 2006. Plant thioredoxins are key actors in the oxidative stress response. *Trends Plant Sci*., 11, 329–334.
15. Szczuka, E., Gielwanowska, I., Leszczuk, A., Domaciuk, M., Pietrusiewicz, J., Bednara, J. 2013. Specific ultrastruc-ture of the leaf mesophyll cells of *Deschampsia antarctica Desv. (Poaceae). Ann. Univ. Mariae-Curie-Sklodowska, sectio C – Biologia*, 68, 25–33.
16. Tarusov B. N., Veselovskii V.A. 1978. *Sverkhslabye svechenyia rastenyi i ikh prykladnoe znachenie.* Moskva: Izdatelsvo MHU, 151.
17. Zuciga, G. E., Alberdi, M., Corcuerm, L. 1996. Non-structural carbohydrates in *Deschampsia antarctica Desv.* from South Shetland islands, Maritime Antarctic. *Environ. Experim. Botany*, 36. 4., 393–399.
18. Zunica-Feest, A., Inostroza, P., Vega, M., Bravo, L., Corcuera, L.J. 2003. Sugars and enzyme activity in the grass *Deschampsia antarctica. Antarctic Science*, 15, 483–491.