ANNALES UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA LUBLIN – POLONIA

VOL. LXVIII, 2 SECTIO C 2013

EWA SZCZUKA¹, IRENA GIEŁWANOWSKA^{2,3}, AGATA LESZCZUK¹, MARCIN DOMACIUK¹, JACEK PIETRUSIEWICZ¹, JÓZEF BEDNARA¹

¹Department of Plant Anatomy and Cytology, Maria Curie-Skłodowska University Akademicka 19, 20-033 Lublin, Poland

²Department of Plant Physiology and Biotechnology, University of Warmia and Mazuria Oczapowskiego 1A, 10-719 Olsztyn, Poland

³Department of Antarctic Biology, Polish Academy of Sciences, Ustrzycka10/12

Warsaw, Poland ewa.szczuka@poczta.umcs.lublin.pl

Specific ultrastructure of the leaf mesophyll cells of Deschampsia antarctica Desv. (Poaceae)

Ultrastruktura komórek mezofilu liści *Deschampsia antarctica* Desv. (Poaceae)

ABSTRACT

The ultrastucture of mesophyll cells of *Deschampsia antarctica* Desv. (Poaceae) leaves was investigated using the standard method of preparing material for examination in transmission electron microscopy (TEM). The investigated leaves were collected from the Antarctic hairgrass growing in a tundra microhabitat and representing xermorphic morphological and anatomical features. The general anatomical features of mesophyll cells are similar to those in cells of another grass leaves. The observations of the ultrastructure of mesophyll cells have shown that the organelles are located close to each other in a relatively small amount of the cytoplasm or closely adhere to each other. Organelles such as mitochondria, peroxisomes, and Golgi apparatus, as well as osmiophilic materials are gathered close to the chloroplasts. The chloroplast of the mesophyll cells of the *D. antarctica* leaf can form concavities filled with the cytoplasm. Such behaviour and ultrastructure of organelles facilitate exchange/flow of different substances engaged in the metabolic activity of the cell between cooperating organelles.

Keywords: Deschampsia antarctica, Poaceae, ultrastructure, leaf cells, cell organelles

STRESZCZENIE

Przy użyciu standardowej metody przygotowywania materiału, badano ultrastrukturę komórek mezofilu liści *Deschampsia antarctica* Desv. (Poaceae) w mikroskopie elektronowym transmisyjnym (TEM). Badane liście zostały zebrane z okazów śmiałka antarktycznego rosnącego w mikrośrodowisku tundrowym, reprezentujących kseromorficzne cechy morfologiczne i anatomiczne. Ogólne cechy anatomiczne komórek mezofilu są podobne do komórek liści innych traw. Obserwacje ultrastruktury komórek wykazały, że organelle komórek mezofilowych występują blisko siebie w stosunkowo niewielkiej ilości cytoplazmy lub ściśle przylegają do siebie. Organelle takie jak mitochondria, peroksysomy, aparaty Golgiego, a także osmofilne materiały gromadzą się w pobliżu chloroplastów. Chloroplasty komórek mezofilu *D. antarctica* często mają wklęsłości wypełnione cytoplazmą. Takie zachowanie i budowa ultrastrukturalna organelli ułatwia wymianę/przepływ różnych substancji zaangażowanych w aktywność metaboliczną między współdziałającymi organellami komórki.

Slowa kluczowe: *Deschampsia antarctica*, Poaceae, ultrastruktura, komórki liści, organella komórkowe

INTRODUCTION

Antarctica, the seventh and the last discovered (commonly accepted data 1820), is the coldest, driest, and windiest of all continents. Although it is the fifth-largest continent in terms of area, very specific, severest and the most inhospitable environment conditions limit the occurrence of angiosperm plants to the coastal region of the Antarctic peninsula and islands. In the Antarctic conditions, only perfectly cold-adapted organisms like algae, animals (for example mites, nematodes, penguins, seals and tardigrades), bacteria, fungi, tundra plants, and protista can survive. Among plants, there are only two unique native species representing angiosperms belonging to the *Caryophyllaceae* and *Poaceae* families. Those species are respectively *Colobanthus quitensis* Bartl. and *Deschampsia antarctica* Desv. occupying among others strongly differentiated microhabitats in the Admiralty Bay region on King George Island (9). The third species of angiosperms, *Poa annua* was introduced to this area by man; therefore, it is of anthropogenic origin.

The object of the research presented in this paper, i.e. the Antarctic hairgrass, is a frequent subject of scientific inquiry. This species is in the focus of interest of a wide range of researchers like ecologists, taxonomists, morphologists, anatomists, embryologists, physiologists, biochemists, and molecular biologists (2, 3, 6, 7, 8). Due to the technical difficulties related to the necessity of rapid fixation and embedding in resins the plant material freshly collected in the natural habitat, the investigation of ultrastructural features of D. antarctica has been neglected for a long time or abandoned after a few modest attempts. Among the few examples, the investigations reported by Romero at al. (1999) on the morphology and anatomy of both native Antarctic angiosperms C. quitensis and D. antarctica should be mentioned. By comparing individuals growing in nature and laboratory results obtained, the authors indicated distinct morphological and anatomical differentiation between them. In turn, Barcikowski et al. (2003) carried out a study on the morphology and anatomy of the Antarctic hairgrass, which had a preliminary character. The time-consuming research on the ultrastructural characteristics of D. antarctica mesophyll cells provided interesting data on the behaviour of the cell organelles in these cells. The observations of the organs of grass individuals growing in the Antarctic tundra and under greenhouse conditions showed significant differences in the anatomical structure, in comparison to plants collected in the wet microhabitat. The differences in the anatomy of different plant organs also reflected noticeable differences in particular cells and tissues.

This paper is focused on the ultrastructure and behaviour of organelles in mesophyll cells of *D. antarctica* growing under the natural habitat of the Antarctic tundra and is a condensed addition to our previous studies.

MATERIAL AND METHODS

Plant material

The green, tiny leaves of *D. antarctica* Desv. (Poaceae) were dissected cautiously from individuals growing in the Antarctic tundra. An area of occurrence of the Antarctic hairgrass located in the close vicinity of the H. Arctowski Antarctic Station was chosen for collection. This Polish Polar station is established on King George Island in the archipelago of the South Shetland Islands (Fig. 1). Plant material was collected by one of the authors, participant of the 34th Polish Research Expedition to Antarctica, Prof. UWM Irena Giełwanowska during Antarctic summer 2010.

Method

The standard method of preparing material for examination in transmission electron microscopy (TEM) was used in the presented investigations. Detailed information can be found in the previous papers (4, 5).

RESULTS AND DISCUSSION

The specific Antarctic climatic conditions render the area in the close vicinity of the Polish Antarctic Station highly differentiated. Even in a relatively short distance from the buildings (about 100 m) the environmental conditions vary significantly enough to distinguish several separate microhabitats. Our research focuses on slightly higher lying areas evidently exposed to extremely unfavourable effects of a range of factors including solar radiation and strong wind throwing sharp crumbs of rock and ice, or pressed by a thick, heavy, and moving snow cover. The area looks inhospitable even in the growing season (Fig. 2). Antarctic hairgrass individuals growing in these sites are the smallest of all the representatives of other microhabitats. Moreover, such plants showed strong xeromorphic features (1, 4, 5).

The mesophyll cells of *D. antarctica* leaves are surrounded by a relatively thick cell wall visible in TEM as a lightly grey layer. Its structure seems to be rather uniformly homogeneous. The boundary space of the cell is filled with a dense cytoplasm. Noteworthy is the fact that the amount of the cytoplasm is very small. It seems to be pressed by the vacuole occupying the central part of the cell. Chloroplasts are immersed in the cytoplasm. In the younger cells, as seen in Figure 3, the chloroplast interior is filled with randomly arranged stacks of thylakoids. The chloroplasts are almost always accompanied by organelles like mitochondria adjacent to their outer membrane. The big, central vacuole is filled with granular material.

In older cells, the ultrastructure of chloroplasts is significantly changed (Fig. 4). Starch grains appeared inside them (the upper cell in the figure). In

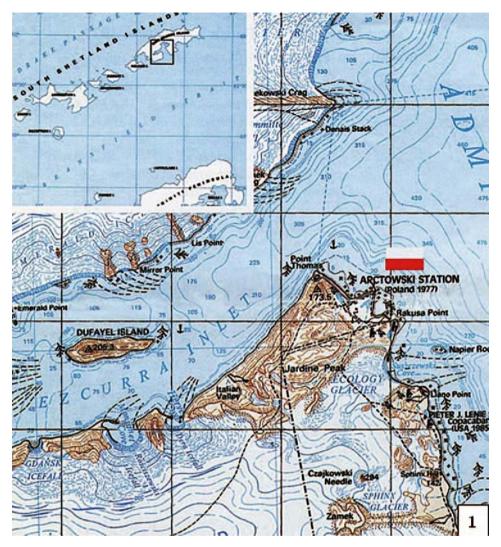


Fig. 1. Location of H. Arctowski Polish Antarctic Station on King George Island. Note the numerous Polish accents in the shown area

some cells, chloroplasts that are regular and elongated in younger cells take a rhomboidal shape (in the cross section). The crowded, squeezed chloroplasts fill almost the entire cell, leaving only a small, central space for the vacuole. The amount of visible grana diminished drastically, especially in the cells filled with numerous starch grains (the lower cell in the figure). In such cells, the vacuoles are filled with a liquid that is almost translucent for the electrons.

The chloroplasts in the mesophyll cells of the Antarctic hairgrass leaves are usually gathered close to each other. In younger cells, the regularly built

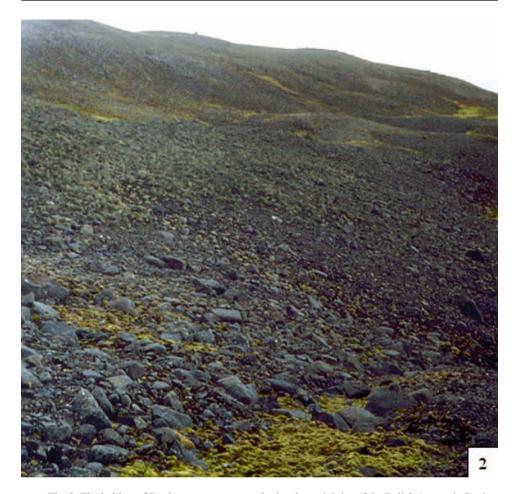


Fig. 2. The habitat of *Deshampsia antarctica* in the close vicinity of the Polish Antarctic Station

chloroplasts are adjacent to each other and to the other organelles. For example, the mitochondria can be squeezed between the chloroplasts, as shown in Figure 5. In this figure, the mitochondrion surrounded by two membranes showed an ultrastructure characteristic of the organelle in the low-activity state. The inner membrane forms a few small cristae. Single mitochondria appear rather sparsely near the chloroplast outer membrane. A few organelles are usually gathered together (Fig. 7). Moreover, based on their ultrastructure, they can be in the high-activity state. Except the mitochondria, the other organelles such as peroxisomes and dictyosomes were observed to exhibit a tendency towards gathering close to the chloroplasts (Fig. 8).

The ultrastructure and behaviour of the organelles described above were observed on sections of mesophyll cells at different lengths of leaves. Based

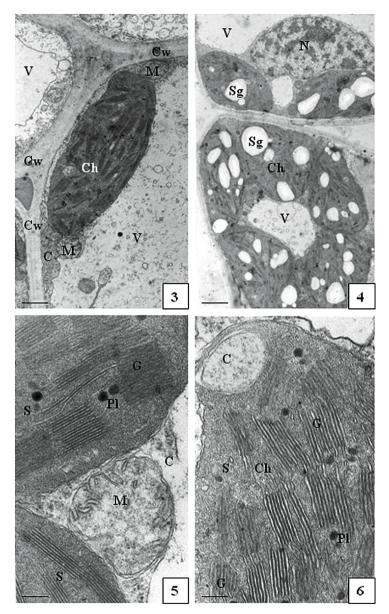


Fig. 3. Fragments of neighbouring *D. antarctica* mesophyll cells. Note the narrow strand of the cytoplasm with elongated chloroplast accompanied by mitochondria. TEM. Scale bar = $1 \mu m$

Fig. 4. Two mesophyll cells with chloroplasts filled with starch grains in the chloroplast stroma. TEM. Scale bar = $0.5~\mu m$

Fig. 5. Portions of two chloroplasts with an adjacent mitochondrion. Note the ultrastructure of the mitochondrion. TEM. Scale bar = $0.3~\mu m$

Fig. 6. Fragment of a chloroplast with a "pocket" filled with the cytoplasm in its upper part. TEM. Scale bar = $0.2~\mu m$

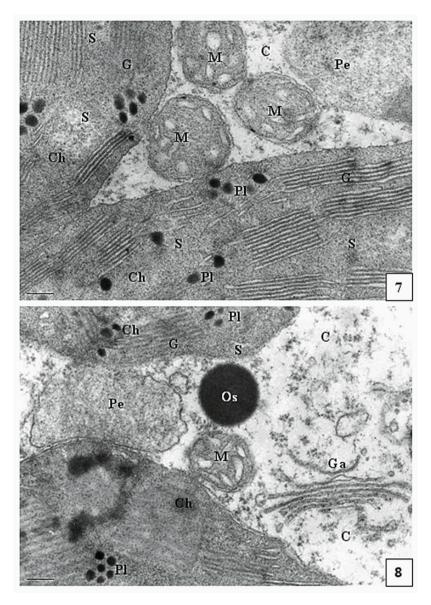


Fig. 7. Deshampsia antarctica mesophyll cell. Fragments of two chloroplasts accompanied by three mitochondria and a peroxisome. Note the atypical ultrastructure of the mitochondria with wide, swollen cristae. TEM. Scale bar = $0.3 \mu m$

Fig. 8. Portion of the mesophyll cell cytoplasm with a peroxisome, mitochondrion, Golgi apparatus, and osmiophilic, globular material gathered close to two chloroplasts. TEM. Scale bar = 0.3 m

C-cytoplasm, M-mitochondrion, Ch-chloroplast, V-vacuole, Cw-cell wall, N-nucleus, S-stroma, Pl-plastoglobules, G-granum, Pe-peroxisome, Sg-starch grain, Ga-Golgi apparatus, Os-osmiophilic material

on our observations, we can conclude that organelles such as chloroplasts, mitochondria, peroxisomes, Golgi apparatus and even osmiophylic materials in the mesophyll cells behave in a specific manner. Moreover, their behaviour, as in the case of mitochondria, shows independence from the activity state of organelles. The mitochondria with narrow cristae (shown in Fig. 5) and with enlarged cristae (visible in Fig. 7) exhibited a tendency towards gathering together in the cytoplasm. This result supports the view presented earlier and concerning the behaviour of organelles in the mesophyll cells of D. antarctica leaves (4, 5). Besides the gathering and formation of groups in the cell cytoplasm, organelles such as mitochondria and plastids/chloroplasts came into close contact. The evident symptom of such a contact was the very often-observed disintegration of the organelle membranes. Such membrane disintegrations make the exchange/flow of different substances engaged in the cell metabolic activity between cooperating organelles easier or faster. Concavities in the chloroplast were sometimes observed. They were usually located at the edge of the chloroplasts near their poles. The content of such a concavity (Fig. 6) had a density and structure similar to the cytoplasm surrounding the chloroplast. Up to now, the cause of such irregularity in the chloroplast structure is unknown, but the explanation of this event will be a challenge for our future investigations. For now, it can only be assumed that the irregularities and pockets enlarged the surface for material exchange between the cytoplasm and organelles.

REFERENCES

- Barcikowski A., Czaplewska J., Loro P., Łyszkiewicz A., Smykla J., Wojciechowska A. 2003. Ecological variability of *Deschampsia antarctica* in the area of Admiralty Bay (King George Island, Maritime Antarctic). In: Problems of Grass Biology (ed.: Ludwik Frey). W. Szafer Institute of Botany, Polish Academy of Sciences, Kraków, 383–396.
- 2. Bokhorst S., Huiskes A., Convey P., Aerts R. 2007. The effect of environmental change on vascular plant and cryptogam communities from the Falkland Islands and the Maritime Antarctic. BMC Ecology DOI: 10.1186/1472-6785-7-15.
- 3. Chwedorzewska K. J., Giełwanowska I., Szczuka E., Bochenek A. 2008. High anatomical and low genetic diversity in *Deschampsia antarctica* Desv. From King George Island, the Antarctic. Polish Polar Research, vol. 29 (4): 377–386.
- 4. Giełwanowska I., Szczuka E. 2005. New ultrastructural features of organelles in leaf cells of *Deschampsia antarctica* Desv. Polar Biology DOI 10.1007/s003 300-005-024-2
- 5. Giełwanowska I., Szczuka E., Bednara J., Górecki R. 2005. Anatomical features and ultrastructure features of *Deschampsia antarctica* (Poaceae) leaves from different growing habitats. Annals of Botany 96: 1109–1119. DOI: 10.1093/aob/mci262
- Krywult M., Smykla J., Wincenciak A. 2003. Influence of ornitogenic fertilization on nitrogen metabolism of the Antarctic vegetatation. In: Olech (ed.). The Functioning of Polar Ecosystems as Viewed Against Global Environmental Changes. XXIX International Polar Symposium, Kraków, 19–21 września 2003, 79–84.

- Lewis Smith R. I. 2003. The enigma of *Colobanthus quitensis* and *Deshampsia antarctica* in Antarctica. In: A. H. I. Huickes, W. W. C. Gieskes, R. L. M. Schorno, S. M. van der Vies, W. I. Volff. (eds). Antarctic Biology in a Global Context. Backham Publ., Leiden, The Netherlands, 234–239.
- 8. Nędzarek A., Chwedorzewska K. J. 2004. Stężenie nutrientów w wodzie zasilającej wybrane stanowiska trawy *Deshampsia antarctica* (King George Island, Antarctica). Folia Universitatis Agriculturae Stetinensis 234: 299–304.
- Parnikoza I., Convey P., Dykyy I., Trokhymets V., Milinevsky G., Tyschenko O., Inozemtseva D., Kozeretska I. 2009. Current status of the Antarctic herb tundra formation in the Central Argentine Islands. Global Change Biology 15: 1685–1693, DOI 10.1111/j.1365-2486.2009.01906.x
- 10. Romero M., Casanova A., Iturra G., Reyes A., Montenegro G., Alberdi M. 1999. Leaf anatomy of *Deshampsia antarctica* (Poaceae) from the Maritime Antarctic and its plastic response to changes in the growth conditions. Revista Chilena de Historia Natural 72: 411–425.