



Morphological and Ultrastructural Changes of Organelles in Leaf Mesophyll Cells of the Arctic and Antarctic Plants of Poaceae Family Under Cold Influence

Authors: Giełwanowska, Irena, Pastorczyk, Marta, Kellmann-Sopyła, Wioleta, Górniak, Dorota, and Górecki, Ryszard J.

Source: Arctic, Antarctic, and Alpine Research, 47(1) : 17-25

Published By: Institute of Arctic and Alpine Research (INSTAAR), University of Colorado

URL: <https://doi.org/10.1657/AAAR0014-019>

BioOne Complete (complete.BioOne.org) is a full-text database of 200 subscribed and open-access titles in the biological, ecological, and environmental sciences published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Complete website, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/terms-of-use.

Usage of BioOne Complete content is strictly limited to personal, educational, and non - commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

Morphological and ultrastructural changes of organelles in leaf mesophyll cells of the Arctic and Antarctic plants of Poaceae family under cold influence

Irena Gielwanowska^{1,2,4}

Marta Pastorczyk¹

Wioleta Kellmann-Sopyła¹

Dorota Górniak³ and

Ryszard J. Górecki¹

¹Department of Plant Physiology, Genetics and Biotechnology, University of Warmia and Mazury in Olsztyn, Oczapowskiego

¹A, 10-719 Olsztyn, Poland

²Institute of Biochemistry and Biophysics PAS, Department of Antarctic Biology and Polish Antarctic Station "H.Arctowski," Ustrzycka 10/12, 02-141 Warsaw, Poland

³Department of Microbiology, University of Warmia and Mazury in Olsztyn, Oczapowskiego 1A, 10-719 Olsztyn, Poland

⁴Corresponding author:

i.gielwanowska@uwm.edu.pl

Abstract

The mesophyll cells of four species of Poaceae flowering plants growing in polar regions were studied—*Deschampsia antarctica* Desv. from the region of the Admiralty Bay on King George Island (West Antarctica) and *D. alpina* (L.) Roem. Sch., *Poa alpina* L. var. *vivipara* and *P. arctica* R. Br. var. *vivipara* from the Hornsund region of Spitsbergen island (Arctic). Ultrastructural changes were analyzed in the organelles of plants growing in Arctic and Antarctic habitats and plants grown in greenhouse, including plants exposed to short-term cold stress. The cell organelles were characterized by structural dynamics. Their morphological plasticity was manifested by elongation, formation of protrusions in the direction of adjacent organelles, as well as cytoplasm-filled pockets and invaginations that increase the contact area and reduce the distance between cell compartments. *D. antarctica* and *P. alpina* var. *vivipara* plants were characterized by highly dynamic cell nuclei with invaginations of the nuclear membrane filled with cytoplasm and organelles, high morphological plasticity, and conformational dynamics of chloroplasts and mitochondria, manifested by variations in the electron-optical density of matrix, membranes, and envelopes. The above could suggest that the studied taxa and their metabolic mechanisms had adapted to severe climates and changing conditions of the polar regions.

DOI: <http://dx.doi.org/10.1657/AAAR0014-019>

Introduction

Low temperature is one of the key environmental stressors that inhibit plant growth in polar regions. The mechanisms that are most susceptible to a rapid drop in temperature are cytoplasmic streaming and photosynthesis, including thylakoid function in chloroplasts. Chloroplasts are the most sensitive organelles, and low temperature is one of the most common abiotic factors that modify chloroplast ultrastructure (Kratsch and Wise, 2000). The changes induced in chloroplasts by various stressors resemble the ultrastructural modifications that are observed in aging cells (Yen and Yang, 1998; Mostowska, 1999; Kołodziejek et al., 2003).

Under the influence of low temperature, chloroplasts become round and cushion-like, and their internal membranes begin to disintegrate gradually. Thylakoids swell, and the complete decay of grana may be observed in extreme cases. Grana thylakoids, which are more sensitive than stroma thylakoids, are the first to undergo disintegration, and their decay is potentiated by reactive oxygen species that are produced in cells under stress (Holá et al., 2008). Membrane decay is accompanied by an increase in the number of plastoglobules in chloroplast stroma, a gradual decrease in the size of starch granules and their ultimate disappearance (Mostowska, 1999).

Studies performed with the involvement of various microscopic techniques revealed significant plasticity of chloroplasts in several species of polar and alpine plants (Lütz et al., 2012), manifested by their ability to produce various deformations. Those deformations take the shape of thin and long protrusions known as stromules (Shaw and Gray, 2011); pockets filled with cytoplasm, organelles and vesicles (Gielwanowska et al., 2005; Gielwanowska and Szczuka, 2005); and differently shaped areas that are free of

thylakoids and are filled only with stroma (Holzinger et al., 2007; Hanson and Sattarzadeh, 2008, 2011).

The mechanisms responsible for plastid deformation and stromule movement in the cytoplasm have not yet been explained. It is believed that cytoskeletal elements could participate in the formation and stabilization of protrusions (Gray et al., 2001). The endoplasmic reticulum probably aids chloroplast protrusions in the process of transporting metabolites and signal molecules (Schattat et al., 2011). Ultrastructural analyses of cells exposed to cold stress confirmed the presence of peripheral endoplasmic reticulum that increases the contact area across which metabolites are transported (Kratsch and Wise, 2000).

Mitochondria are considered to be more stable and more resistant to cold stress than chloroplasts. Studies of model plants revealed no changes in mitochondrial ultrastructure even during advanced chloroplast decay (Nessler and Wernsman, 1980). Experiments performed on plants with low tolerance to cold stress demonstrated that mitochondrial swelling and disorganization were visible already within several hours of exposure to stress (Leddet and Geneves, 1982). Other symptoms of mitochondrial damage included elongated crests and vacuolization (Ishikawa, 1996).

The effects of cold stress on the cell nucleus have not yet been thoroughly researched, probably because the changes noted in those organelles are not extensive (Kratsch and Wise, 2000). Ishikawa (1996) described enlarged nuclei in bean cells where chromatin, nucleolus, and other high-density cytoplasmic components were fragmented under exposure to cold stress. The expansion of Golgi apparatus vesicles and the endoplasmic reticulum, which is surrounded by a large volume of membrane microvesicles, was observed immediately before chloroplast swelling induced by cold stress (Stefanowska et al., 2002).

This study examined ultrastructural changes in the organelles of mesophyll cells of *D. alpina*, *P. alpina* var. *vivipara*, and *P. arctica* var. *vivipara* grasses native to the Arctic and *D. antarctica* plants native to West Antarctica. Mesophyll cells were analyzed in plants growing naturally in polar regions and in plants grown in an experimental greenhouse and exposed to cold stress.

Materials and Methods

PLANT MATERIAL

Deschampsia antarctica Desv. plants were harvested during polar expeditions organized in 2002–2010 in the region of the Henryk Arctowski Polish Antarctic Station on King George Island (62°09'S and 58°28'W). *Deschampsia alpina* (L.) Roem. Sch., *Poa alpina* L. var. *vivipara*, and *Poa arctica* R. Br. var. *vivipara* plants were harvested in the region of the Stanisław Siedlecki Polish Polar Station in Hornsund, Spitsbergen (77°00'N and 15°33'E). Plant samples were chemically preserved and embedded in Epon 812 epoxide resin at harvest. Seeds and live plants were secured and transported to Olsztyn where the harvested specimens were planted, and seeds were sown and grown at 18–20 °C in the greenhouse of the University of Warmia and Mazury in Olsztyn. The specimens were replanted for restoration purposes, and no other treatments were applied. The established treatments were a source of fresh plant material for laboratory analyses. Plant tissue was sampled for analysis. In this study, those plants are referred to as greenhouse plants grown at 20 °C. Selected specimens were additionally grown in a climate test cabinet (Nüve Climate Test Cabinet, TK 252) at a temperature of 8/4 °C (day/night) and 16/8 h (day/night) photoperiod. Tissue samples were also collected from the above plants for analysis. In this study, those plants are referred to as plants grown at 8/4 °C.

PREPARATION OF SPECIMENS FOR ANALYSIS UNDER A LIGHT MICROSCOPE (LM) AND A TRANSMISSION ELECTRON MICROSCOPE (TEM)

Specimens for LM and TEM analysis were obtained from the central part of healthy green leaf blades (second or third leaf) and were fixed in 3.5% glutaraldehyde solution in phosphate buffer with the pH of 7.0 for 12 h at room temperature, followed by secondary fixation in 2.5% osmium tetroxide solution. The specimens were rinsed and dehydrated in a graded series of alcohols and acetone, and were embedded in Poly Bed 812 epoxide resin (Polyscience). Microtome sections were prepared in the Leica Ultracut R microtome using Diatome diamond knives. Semi-thin sections, 1.5 µm thick, were placed on slides and stained with toluidine blue and azure B. The specimens were mounted with a drop of glycerin. They were analyzed under the Nikon Eclipse 80i light microscope with compatible hardware and software (NIS ELEMENTS) for digital image recording. Ultra-thin sections, 60–90 nm thick, were mounted on nickel grids with 300 mesh squares. Immediately before examination, saturated aqueous uranyl acetate solution and lead citrate were added to impart contrast to the specimens. The specimens were examined and electronograms were obtained simultaneously under two transmission electron microscopes—JEOL JEM 100S and JEOL 1400. JEOL JEM 100S supports analog image recording, whereas JEOL 1400 is equipped with hardware and iTEM software for recording data files.

ANALYSIS OF CELL ORGANELLE RESPONSES TO SHORT-TERM COLD STRESS

Deschampsia antarctica and *Poa alpina* var. *vivipara* plants grown in pots in a greenhouse (one plant per pot, two replications) were transferred to the laboratory and acclimatized. After 24 h of incubation at room temperature (18–20 °C), the pots were placed in the Heraeus BK6160 incubator (Kendro Laboratory Products) with a constant temperature of 20 °C and 20/4 h (day/night) photoperiod. After 24 h, the plants were subjected to short-term cold stress at 4 °C for 48 h. After 1, 4, and 12 h, 3- to 4-mm-long leaf segments (central part of the leaf blade) were cut off and fixed. After 48 h of exposure to cold stress, the plants were heated to 20 °C, and the last tissue samples were collected after 24 h. Tissue samples collected from plants at 20 °C served as the control.

Results

STRUCTURAL CHANGES IN THE ORGANELLES OF LEAF MESOPHYLL CELLS FROM PLANTS OF THE GENUS DESCHAMPSIA

Circular and ellipsoid mesophyll cells were observed under a transmission electron microscope (Fig. 1, parts a, g–j). They were characterized by dense cytoplasm with numerous vesicles (Fig. 1, parts b–f) as well as foamy cytoplasm (Fig. 1, part h). The internal membrane system was well developed with visible Golgi apparatuses (Fig. 1, parts k, go) and endoplasmic reticulum cisternae, possibly a part of the trans-Golgi network (Fig. 1, parts k–m).

The mesophyll cells of the analyzed plants of the genus *Deschampsia* had a typical structure with major organelles, including nuclei, chloroplasts, mitochondria, peroxisomes, Golgi apparatuses, and endoplasmic reticulum. Densely adhering organelles were observed in the cells of plants harvested in the Arctic and Antarctica and plants grown in a greenhouse under varied conditions. In most cases, numerous, densely packed chloroplasts in mesophyll cells formed a dense layer along the cell wall (Fig. 1, parts g, j, m). Those chloroplasts were short and round, and they had dense stroma and well-developed grana thylakoids. Grana stacks comprising numerous thylakoids were connected by a system of intergrana thylakoids. Small, strongly osmiophilic lipid plastoglobules were visible between thylakoids. The mesophyll cells of *D. antarctica* plants harvested from their natural habitat were characterized by atypical chloroplast surfaces, including membrane invaginations filled with cytoplasm (Fig. 1, part d) as well as shorter (Fig. 1, part d) and longer (Fig. 1, part e) protrusions filled only with stroma. The protrusions had equal width or were wider at the ends (Fig. 1, parts d, e), they were adjacent to the cell wall and were prolonged in the direction of the nearest organelle.

The cells of plants harvested from the Arctic and Antarctica and plants grown in a greenhouse contained mitochondria with regular oval or elongated shape (Fig. 1, parts b, d, m; Fig. 2, parts a, mi) as well as atypically shaped mitochondria. In the mesophyll cells of *D. alpina* plants, very long (up to 10 µm) mitochondria were observed near chloroplasts distributed along the cell wall and near the cell nucleus (Fig. 2, parts g–i). Long mitochondria of equal width or greater width at the ends were observed in the cytoplasmic space between the cell nucleus and chloroplasts in *D. antarctica* plants (Fig. 2, g–j). Large spherical and oval (Fig. 1, parts c, f, pe) or elongated (Fig. 1, parts m, pe) peroxisomes were noted in the vicinity of mitochondria and chloroplasts.

In the mesophyll cells of plants of the genus *Deschampsia*, the nucleus was generally positioned in the center of the cytoplasmic matrix (Fig. 1, parts a, h; Fig. 2, parts a, b, d; Fig. 3, part

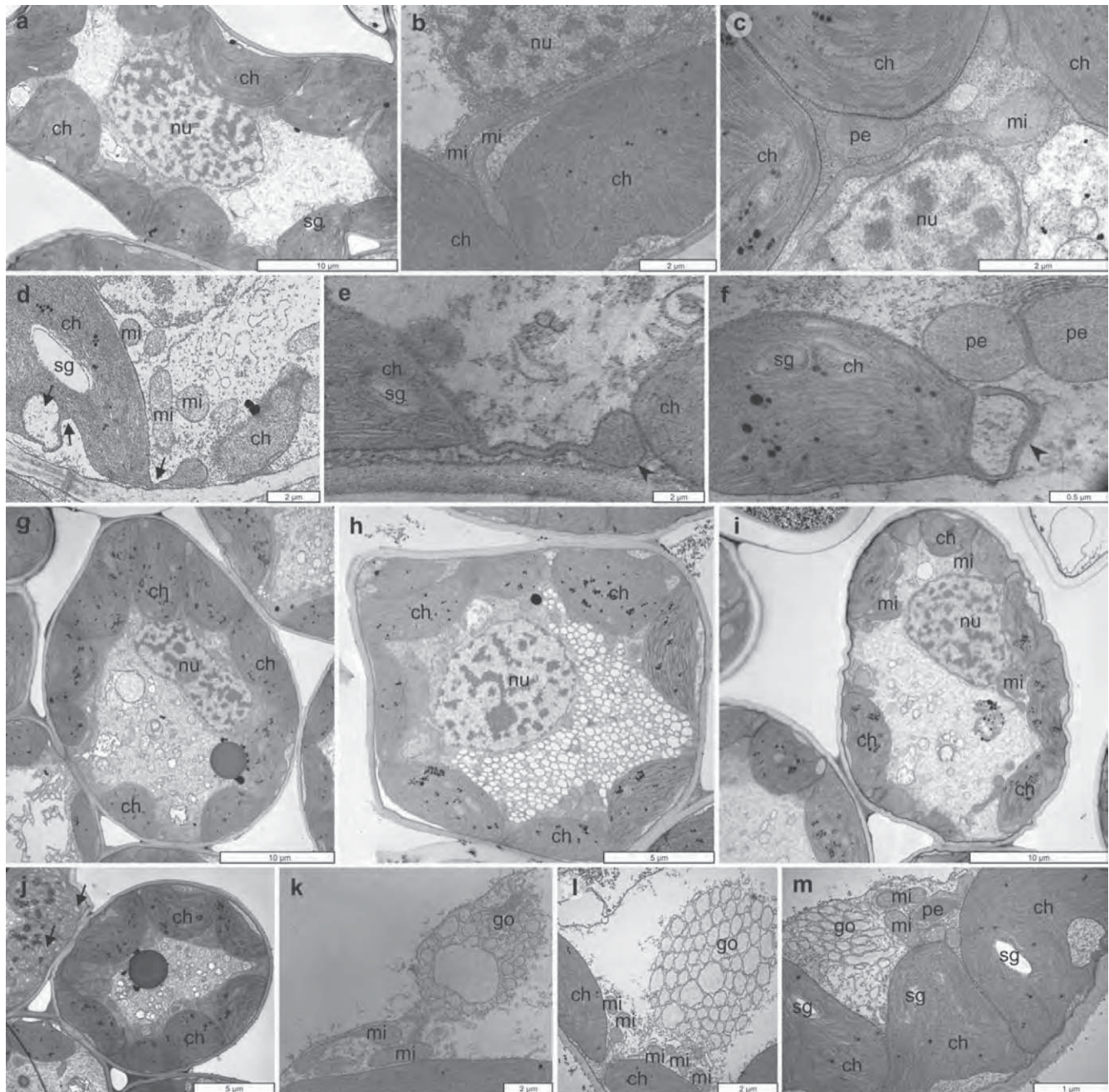


FIGURE 1. Ultrastructure of leaf mesophyll cells of *Deschampsia antarctica* grown in the Antarctic (a–f), grown under greenhouse conditions at the temperature of 20 °C (g–i), and grown at a variable temperature of 8/4 °C (j–m). (a) Short and thick chloroplasts are tightly packed in the cytoplasm region near the cell wall. (b and c) Atypical mitochondria in mesophyll cells of *D. antarctica*. A highly elongate mitochondrion, with even thickness in the central section (b), in the cytoplasmic space between the nucleus, chloroplasts and the peroxisome (pe). (d–f) Modified organelles in leaf mesophyll cells of *D. antarctica*. Chloroplasts feature surface invaginations (a, arrows) and long protrusions without thylakoids (arrowheads) toward the nearest organelles. Mitochondria (mi) and peroxisomes (pe) are positioned next to the chloroplasts (ch), often in the direct vicinity. (g–i) Cells of *D. antarctica*. Thick, differentiated cytoplasm with vesicles can be seen in Figure 1, parts g and i, foamy cytoplasm is shown in Figure 1, part h. (j–m) Mesophyll cell and fragments of mesophyll cells protoplasts in plants of *D. antarctica* with chloroplasts (ch), mitochondria (mi), peroxisomes (pe), and the Golgi apparatus (go). In Figure 1, part j, a cell with well-organized cytoplasm without signs of organelle degradation and a fragment of a cell with disorganized chloroplasts are shown (arrows). An abundance of plastoglobules and dilated thylakoid lumina can be observed in those chloroplasts. In Figure 1, parts k–m, chloroplasts contain starch grains and infrequent plastoglobules. Large peroxisomes (m, pe) can be observed near differently shaped mitochondria. A fragment of the Golgi apparatus (go) with vesicles and cisternae is shown.

g) or, less frequently, toward the side of the cell (Fig. 1, parts g, i; Fig. 2, part i; Fig. 3, parts a, b, h). Most cell nuclei were oval in shape (Fig. 1, parts a–h), but nuclei with centrally located V-

shaped invaginations (Fig. 2, part a) filled with cytoplasm with diluted material and vesicles, nuclei with dense cytoplasm with mitochondria (Fig. 2, parts d, e), as well as nuclei with enclosed

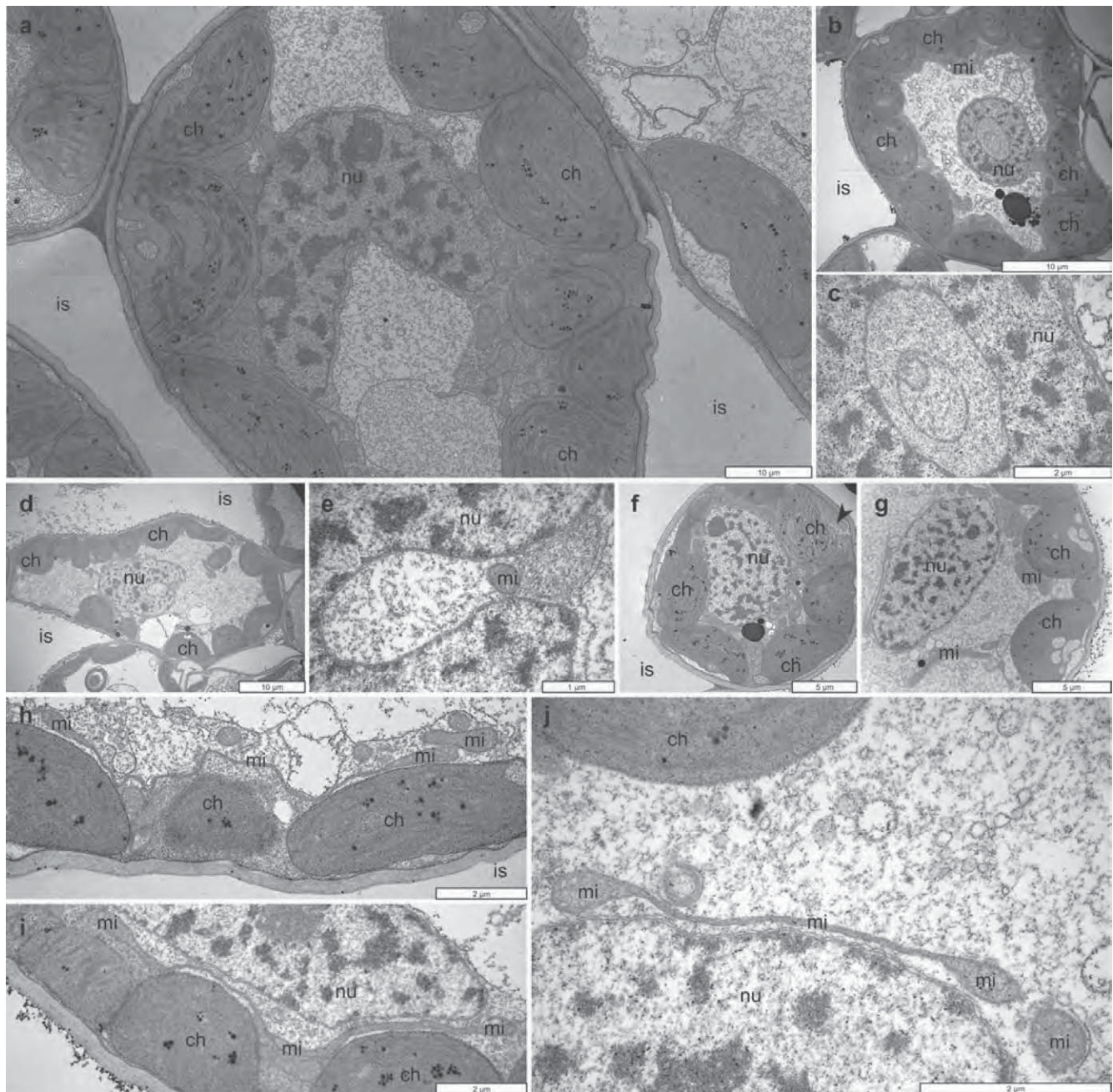


FIGURE 2. Ultrastructure of mesophyll cells of *Deschampsia antarctica* (a–c) and *Deschampsia alpina* (d–j) grown under greenhouse conditions. (a–c) Mesophyll cells near intercellular spaces (is). Short and thick chloroplasts near the cell wall contain numerous osmophilic plastoglobules. V-shaped cell nucleus (a, nu) in the central part of the cell. A *Deschampsia antarctica* cell with a nucleus and a centrally positioned cytoplasm region bound by plasmalemma is shown in Figure 2, parts b and c. (d and e) A *Deschampsia alpina* cell with a centrally positioned nucleus (nu) with a characteristic invagination filled with cytoplasm and mitochondria (mi). (f) A cell of *Deschampsia alpina* grown under greenhouse conditions. Degenerative changes are visible in one of the chloroplasts (arrowhead). (g–j) Atypical mitochondria in mesophyll cells of *Deschampsia alpina*. Elongate mitochondria near the cell nucleus (Fig. 2, part g), chloroplasts (ch) and mitochondria between cell compartments. Highly elongate mitochondrion (mi), reaching 10 µm in length, wider towards the ends, are stretched along three chloroplasts (ch) near the cell wall (Fig. 2, part h). Elongate mitochondrion stretched between the cell nucleus and four chloroplasts (Fig. 2, part i).

cytoplasmic matrix and vesicles separated by the plasma membrane (Fig. 2, parts b, c) were also observed. All cell nuclei in plants of the genus *Deschampsia* contained large quantities of evenly distributed heterochromatin.

Mesophyll cells of *D. alpina* and *D. antarctica* plants harvested from natural habitats and grown in a greenhouse had typical cytoplasmic organization with healthy organelles, but in some cases the symptoms of degenerative changes were observed. Dilated thylakoid

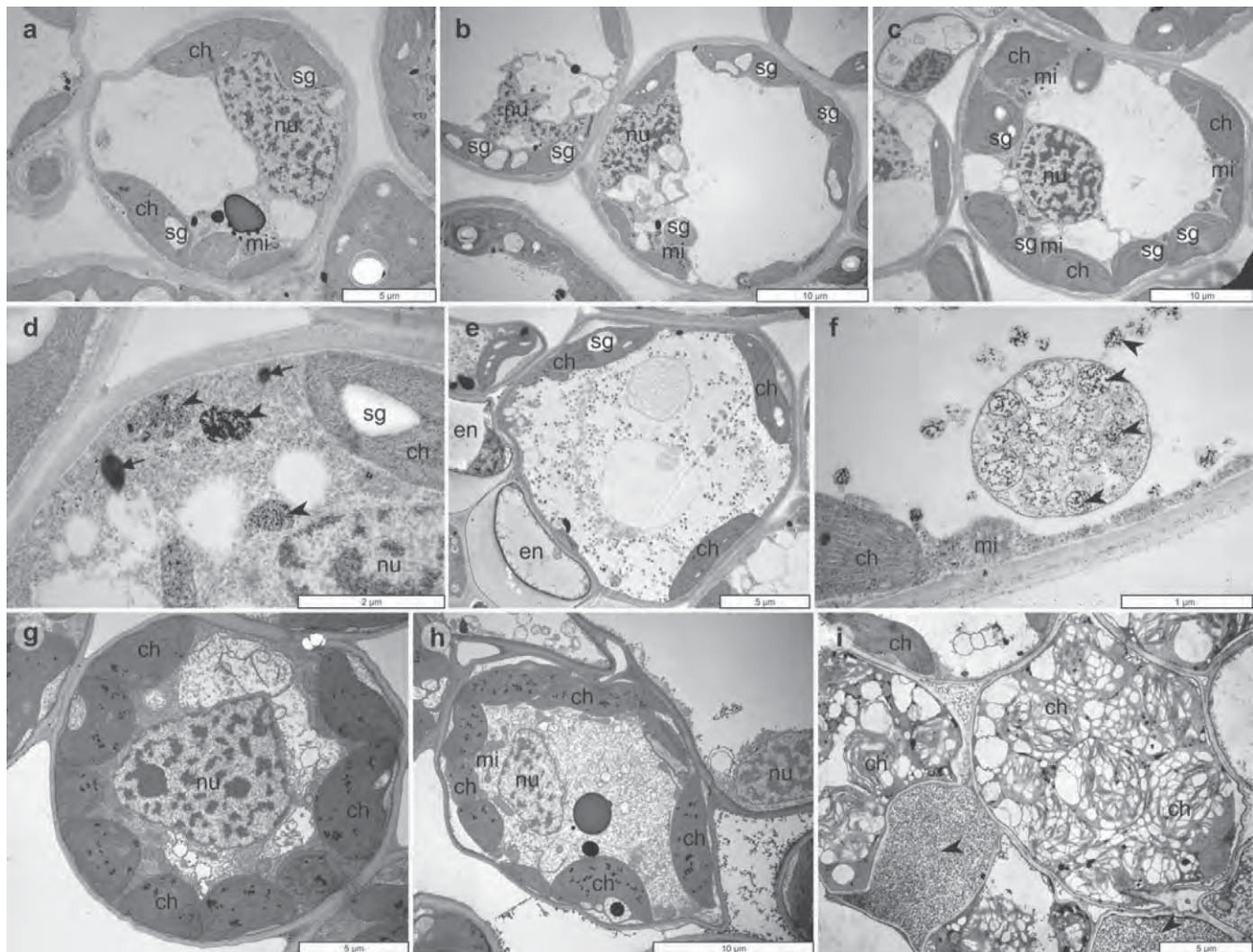


FIGURE 3. Changes in mesophyll cell protoplasts of *Deschampsia antarctica* (a–f and i) and *Deschampsia alpina* (g, h) grown under greenhouse conditions at a variable temperature of 8/4 °C (g, h), exposed to lower temperature for 4 h (a–d) and 12 h (e, f). Ultrathin sections were visualized by TEM. (a) Mesophyll cells with chloroplasts (ch) containing large individual starch grains (sg). Lipid globules are fused in the cytoplasm. (b) The cell have deformed nuclei (nu). (c and d) Mitochondrial regions (arrowheads) contain various quantities of electron-dense granular material which differs from that observed in the cytoplasm (arrow). (e and f) A disorganized and degraded mesophyll cell protoplast. The protoplast contains grains with electron-dense material (arrowheads). Electron-dense plasmalemma is visible in endoderm cells (en), but is not clearly differentiated in mesophyll cells. (g and h) Mesophyll cells of *Deschampsia alpina* grown under greenhouse conditions at a variable temperature of 8/4 °C. Short and thick chloroplasts are clearly visible in Figure 3, part g. (i) Fragment of the leaf mesophyll of *Deschampsia antarctica* with disorganized and degraded protoplasts. Material with granular structure can be observed in the cells and in apoplastic air space (arrowheads).

lumina and plastoglobule clusters were noted in chloroplasts (Fig. 1, part j, arrows). Mesophyll cells of *D. antarctica* with completely decayed protoplasts contained granular material in the apoplast (Fig. 3, part i, arrowheads). Selected cells of *D. alpina* plants grown in a greenhouse and exposed to cold stress contained short and round chloroplasts, including chloroplasts with extended thylakoid lumina and deformed surface (Fig. 2, part f, arrow, arrowhead; Fig. 3, part g), as well as flat chloroplasts forming narrow protrusions filled with stroma (Fig. 3, part h). All chloroplasts contained dense stroma with plastoglobule clusters and infrequent starch granules. Large, merging drops of osmiophilic material (Fig. 3, parts a, d, h) and areas with granular osmiophilic material (Fig. 3, parts c, d, f, arrowheads) were observed in the cytoplasm. The chloroplasts of mesophyll cells of *D. antarctica* plants exposed to cold stress (8/4 °C) contained scarce,

large starch globules, but plastoglobules were not reported (Fig. 3, parts a–d). Those cells also contained deformed nuclei (Fig. 3, parts a, b), and plants exposed to cold stress for 12 h were characterized by disorganized and degraded protoplasts (Fig. 3, parts e, f). The plasma membrane in degraded mesophyll cells was weakly osmiophilic (Fig. 3, parts a–d, f), whereas the plasma membrane of endodermal cells in the same part of the leaf was electron dense and clearly visible (Fig. 3, part e, en).

ULTRASTRUCTURAL CHANGES IN THE ORGANELLES OF LEAF MESOPHYLL CELLS FROM PLANTS OF THE GENUS POA

Plants of the genus *Poa* were characterized by regular oval or ellipsoid mesophyll cells with a different degree of vacuoli-

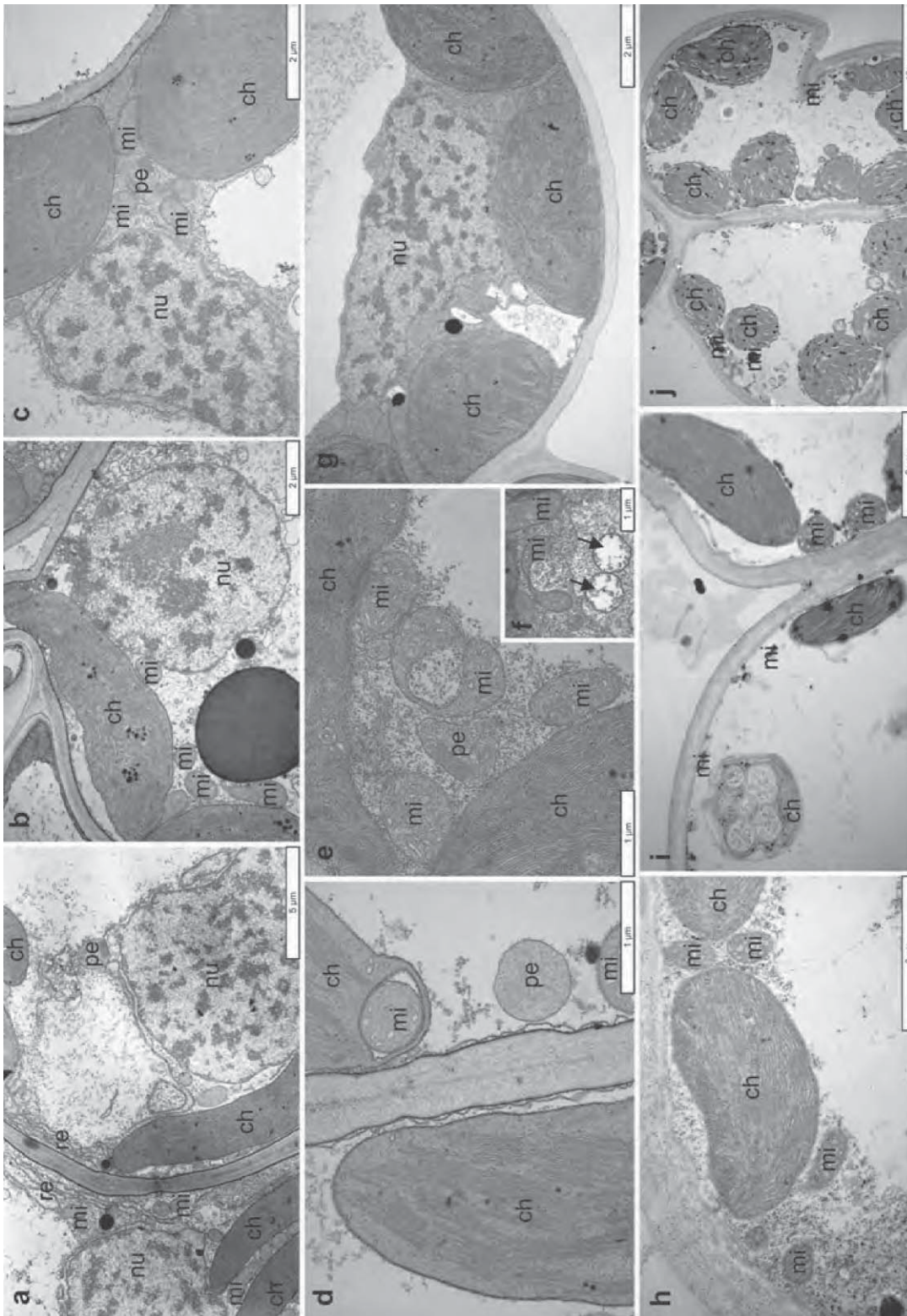


FIGURE 4. Ultrastructure of the leaf mesophyll cells of *Poa* plants. (a and b) A fragment of a leaf mesophyll cell of *Poa alpina* var. *vivipara* grown in the natural habitat of the Arctic. Endoplasmic reticulum (re) near the cell wall, elongate chloroplasts (ch), mitochondria (mi), and peroxisomes (pe) can be observed. (c) A vacuolated mesophyll cell of *Poa alpina* var. *vivipara* grown at a variable temperature of 8/4 °C with chloroplasts (ch), mitochondria (mi), and peroxisomes (pe) near the cell nucleus with a folded envelope. (d) Mesophyll cell of *Poa alpina* var. *vivipara* grown at a variable temperature of 8/4 °C with mitochondrion (mi) inside the chloroplast (ch). (e and f) Mesophyll cell of *Poa alpina* var. *vivipara* grown at a variable temperature in dense cytoplasm and vacuoles formed after the lysis of mitochondria (arrows). (g) A fragment of a leaf mesophyll cell of *Poa arctica* var. *vivipara* grown at a variable temperature of 8/4 °C. The nucleus has irregular surface. Short and thick chloroplasts (ch), mitochondria (mi) in mesophyll cell of *Poa arctica* var. *vivipara* exposed to lower temperature (4 °C) for 12 h. (i and j) Chloroplasts (ch) and mitochondria (mi) in mesophyll cells of *Poa arctica* var. *vivipara* exposed to lower temperature (4 °C) for 4 h. Dilated thylakoid lumina in chloroplasts and the accumulation of electron-dense granular material in mitochondria are shown in Figure 4, part i.

zation. Mesophyll cells contained typical organelles, including nuclei, chloroplasts, mitochondria, peroxisomes, various types of endoplasmic reticulum and dictyosomes (Fig. 4, parts a–h). The cells of *Poa alpina* var. *vivipara* plants harvested in the Arctic contained dense cytoplasm with well-developed peripheral endoplasmic reticulum (Fig. 4, part a, re), elongated chloroplasts, nuclei with irregular surface, and large drops of dense osmophilic material near mitochondria (Fig. 4, part b). The mesophyll cells of *Poa* plants grown at varied temperatures (8/4 °C) featured nuclei with irregular surface and a well-developed system of internal membranes (Fig. 4, part c). Chloroplasts with high structural plasticity densely surrounded the mitochondria (Fig. 4, part d), and numerous bent mitochondria (Fig. 4, parts e, f, mi) were observed. Vacuolizing mitochondria were visible (Fig. 4, part f, arrows). In the mesophyll cells of *Poa arctica* var. *vivipara* plants grown at varied temperatures (8/4 °C), the surface of the nucleus was generally irregular, and short and thick chloroplasts (ch) were distributed in the cytoplasm along the cell wall (Fig. 4, part g). Oval, spherical, or crescent-shaped mitochondria and relatively large peroxisomes were observed between chloroplasts. In addition to mitochondria with a dense matrix and well-developed internal membranes, mitochondria with clearly dilated lumina of the internal membrane system (Fig. 4, mi) were also noted. More advanced changes in organelles and protoplasts were observed in cells exposed to short-term cold stress (4 °C) for 12 h, in particular in chloroplasts and mitochondria with decayed internal membranes. Such chloroplasts did not contain starch, and electron dense granular material was observed in mitochondria (Fig. 4, parts i, j, mi).

Discussion

Numerous research studies have demonstrated that the cells of polar plants are characterized by higher levels of metabolic activity than the cells of plants growing in a temperate climate (Lütz et al., 2012). The existence, individual development, and reproductive patterns of vascular plants native to the Arctic and Antarctica, including *Deschampsia alpina*, *Deschampsia antarctica*, *Poa alpina* var. *vivipara*, and *Poa arctica* var. *vivipara*, indicate that those species are adapted to unsupportive environments and tolerate stress. They have developed multiple adaptive mechanisms of various efficiency for counteracting or fighting the negative consequences of environmental stressors.

MORPHOLOGICAL AND ANATOMICAL RESPONSES OF POLAR PLANTS TO ENVIRONMENTAL STRESSORS

In polar regions, plant species of the family Poaceae grow in dense patches and form compact mats with other species. Those fine, cushion plants are limited in height above ground and develop strong root systems in soil and rock clefts to mitigate the adverse effects of strong winds. Their dense development prevents heat loss and contributes to a positive energy balance in environments characterized by low temperature and powerful winds (Block et al., 2009).

According to many authors, polar plants are xerophytes (Romero et al., 1999; Lewis-Smith, 2003; Parnikoza et al., 2011) whose specific anatomical features support growth and development in extreme habitats. These features include tracheal elements with a small diameter. Research has demonstrated that tracheal elements with a diameter smaller than 30 µm significantly inhibit water freezing (Lütz, 2010).

ULTRASTRUCTURAL AND CONFORMATIONAL DYNAMICS OF ORGANELLES

Metabolic disruptions in cells induced by environmental factors are manifested in the ultrastructure of cell organelles. Cellular components have varied tolerance to excessive light, low temperature and dehydration. Chloroplasts are believed to be the most sensitive organelles (Lütz et al., 2012), whereas cell nuclei, mitochondria, and peroxisomes are characterized by greatest stability (Kratsch and Wise, 2000). The ultrastructural organization of organelles determines plant responses to abiotic stress.

Cells with densely packed organelles were observed in plants harvested from polar regions and plants grown in a greenhouse. Dense arrangement of organelles is noted in all plants, not only species native to the Arctic and Antarctica. Close contacts and metabolic interactions between organelles have been observed in the photorespiration process where chloroplasts, mitochondria, and peroxisomes are distributed in close proximity (Körner and Larcher, 1988). Organelle distribution is determined by the chemical gradient associated with bonds between selected metabolites and macromolecular structures (Bereiter-Hahn and Vöth, 1994). Mitochondria are accumulated near cellular structures and organelles that require energy in the form of adenosine triphosphate (ATP). Those organelles remain in close contact with the nuclear membrane, rough endoplasmic reticulum, cytoplasmic membrane, and chloroplasts (Logan, 2006).

In the analyzed cells of *Deschampsia* and *Poa* plants, mitochondria were characterized by a well-developed system of internal membranes and large membrane surfaces where respiratory enzymes are located. Some mitochondria had a weakly organized system of internal membranes. Numerous, significantly elongated mitochondria of equal width or greater width at the ends were also noted. Their polymorphism could be attributed to variations in the energy balance resulting from different intramitochondrial levels of ATP and adenosine diphosphate (ADP) (Logan and Leaver, 2000, 2006).

In live plant cells, mitochondria are able to change their location and shape through elongation, shrinking, branching, bending, and swelling. The number, size, and distribution of those organelles inside cells are directly affected by constant fusion and fission (Bereiter-Hahn and Vöth, 1994).

In the cells of *Deschampsia antarctica* and *Deschampsia alpina* plants (Fig. 2, parts g–j, mi), elongated mitochondria with wider ends along the long axis could be organelles that were undergoing division due to increased demand for ATP. Circular, ellipsoid, and semi-crescent-shaped mitochondria were also observed. The shape of mitochondria affects their ability to move inside the cell. Differently shaped mitochondria are characterized by various movement patterns, and neighboring mitochondria move independently (Logan and Leaver, 2000; Logan, 2006). Elongated mitochondria are significantly more mobile. Mitochondrial polymorphism and mobility patterns are closely related to the energy status of those organelles (Logan and Leaver, 2000).

Ultrastructural observations of leaf mesophyll cells in several species of vascular plants performed with the involvement of various microscopic techniques revealed different types of chloroplast deformations. In *Oxyria digyna*, those deformations take the form of large, thylakoid-free areas filled only with stroma (Holzinger et al., 2007) as well as characteristic pockets or invaginations filled with cytoplasmic components (Gielwanowska and Szczuka, 2005). Mitochondria and peroxisomes are frequently observed inside pocket extensions. This dense arrangement of cellular organelles probably aids photorespiration and prevents photoinhibition (Holzinger et al.,

2007). The presence of the above structures has been reported in alpine plants, including *Ranunculus glacialis* (Lütz, 1987; Larcher et al., 1997), *Geum reptans*, *Oxyria digyna* (Lütz and Engel, 2007), *Carex curvula*, *Leontodon helveticus*, *Silene acaulis* (Buchner et al., 2007), and in two species native to Antarctica (Gielwanowska et al., 2005). Long and thin stromules extending from the surface of chloroplasts have also been observed (Gray et al., 2001). They were noted in *D. antarctica* plants harvested in Antarctica. In their mesophyll cells, individual chloroplasts simultaneously formed several types of deformations, including pockets, invaginations, and long extensions only with stroma and long extensions with internal membrane system (Gielwanowska et al., 2005).

The role of plastid deformations has not yet been fully explained. Extensions probably increase the contact area and reduce the distance between adjacent organelles. The above aids metabolite flow and eliminates energy expenditure related to long-distance transport. A recent study by Busch et al. (2013) demonstrated that in wheat and rice cells, chloroplasts increase their area by forming stromules that extend to the cell wall. This mechanism expands the contact area between chloroplasts and intercellular spaces, and it facilitates reassimilation of carbon dioxide released during photorespiration and normal respiration.

Individual plastoglobules and plastoglobule clusters in the stroma were observed in all of the studied plant taxa. Those structures preserve the integrity of chloroplast membranes under exposure to oxidative stress (Zamora et al., 2010). Clusters of small lipid plastoglobules were observed in the chloroplasts of disintegrating cells in the analyzed polar plants. They could be formed by the thylakoid membrane on the stromal side or by successive vesicles on the existing plastoglobules (Austin et al., 2006).

Spherical and smooth cell nuclei as well as V-shaped nuclei with numerous grooves and cytoplasm-filled channels were observed in the mesophyll cells of plants of the genus *Deschampsia*. Deep cytoplasmic channels in the nucleus were also noted in pea cells analyzed under a transmission electron microscope (Bowes, 1996) and in onion cells examined under a confocal microscope (Collings et al., 2000).

In addition to active cells with typical cytoplasmic organization, the mesophyll of plants harvested in the Arctic and Antarctica as well as greenhouse-grown plants contained cells with disorganized plastids, mitochondria, and protoplasts. Chloroplasts with dilated thylakoid lumina and numerous clusters of lipid plastoglobules were found in a single cell with healthy chloroplasts. Structural and functional changes in the photosynthetic apparatus, which is found in the unique system of internal membranes, are the first symptoms of mild or moderate stress, and they can be regarded as receptors of environmental stimuli (Kratsch and Wise, 2000; Holá et al., 2008). Dilated thylakoid lumina are the first indicators of damage. In the analyzed cells, chloroplast damage could have been induced by stressors, but their exact nature is difficult to determine.

In addition to cells containing chloroplasts with different levels of activity, cells with completely relaxed internal membranes, and lysed protoplast fragments were also observed. Numerous osmophilic drops and the accumulation of granular material in the apoplast were noted in those cells. The underlying causes of the above are difficult to determine, especially because only fragments of mature, green, undamaged and healthy leaf blades were fixed for TEM analysis. Despite the above, symptoms of cell decay were present. Several authors reported cases of sudden cell disintegration caused by traumatic shock such as sudden freezing of plant tissue (Kratsch and Wise, 2000).

Some cells were characterized by changes resembling programmed cell death (PCD) events (Pennell and Lamb, 1997) that begin with the expression of unique proteins responsible for cell decomposition and self-digestion. This form of cell decay was observed in nutrient-deficient plants (Bassham, 2007), and it is probably common in polar plants.

Ultrastructural comparisons of mesophyll cells in plants growing in lowland, mountainous, and polar regions revealed that those plants had nearly identical structure (Lütz et al., 2012), and the only differences were reported in morphological and specific plasticity and the degree of cooperation between organelles, which probably enables polar plants to rapidly modify their metabolic processes and adapt to unsupportive and changing habitats.

Conclusion

The cell organelles of *Deschampsia alpina*, *Deschampsia antarctica*, *Poa alpina* var. *vivipara*, and *Poa arctica* var. *vivipara* were characterized by unique plasticity. In cells of all species, chloroplasts, mitochondria, and nuclei form long protrusions or concavities, which increase contact surface between neighboring cell compartments. Such features of organelles indicate tight cooperation and highly intense metabolic processes, which may be important for growth of examined plants in harsh environmental conditions that require intimate energy balance.

Acknowledgments

We would like to thank the reviewers for constructive advice, which has improved our paper.

References Cited

- Austin, J. R., Frost, E., Vidi, P. A., Kessler, F., and Staehelin, A., 2006: Plastoglobules are lipoprotein subcompartments of the chloroplast that are permanently coupled to thylakoid membranes and contain biosynthetic enzymes. *The Plant Cell*, 18: 1693–1703.
- Bassham, D. C., 2007: Plant autophagy—more than a starvation response. *Current Opinion in Plant Biology*, 10: 587–593.
- Bereiter-Hahn, J., and Vöth, M. 1994: Dynamics of mitochondria in living cells: shape changes, dislocations, fusion, and fission of mitochondria. *Microscopy Research and Technique*, 27: 198–219.
- Block, W., Smith, L. R. I., and Kennedy, A. D., 2009: Strategies of survival and resource exploitation in the Antarctic fellfield ecosystem. *Biological Reviews*, 84: 449–484.
- Bowes, B. G., 1996: *A Colour Atlas of Plant Structure*. London: Manson Publishing, 192 pp.
- Buchner, O., Holzinger, A., and Lütz, C., 2007: Effects of temperature and light on the formation of chloroplast protrusions in leaf mesophyll cells of high alpine plants. *Plant, Cell and Environment*, 30: 1347–1356.
- Busch, F. A., Sage, T. L., Cousins, A. B., and Sage, R. F., 2013: C₃ plants enhance rates of photosynthesis by reassimilating photorespired and respired CO₂. *Plant, Cell and Environment*, 36: 200–212.
- Collings, D. A., Carter, C. N., Rink, J. C., Scott, A. C., Wyatt, S. E., and Strömberg-Allen, N., 2000: Plant nuclei can contain extensive grooves and invagination. *The Plant Cell*, 12: 2425–2439.
- Gielwanowska, I., and Szczuka, E., 2005: New ultrastructural features of organelle in leaf cells of *Deschampsia antarctica* Desv. *Polar Biology*, 28: 951–955.
- Gielwanowska, I., Szczuka, E., Bednara, J., and Górecki, R., 2005: Anatomic features and ultrastructure of *Deschampsia antarctica* (Poaceae) leaves from different growing habitats. *Annals of Botany*, 96(6): 1109–1119. <http://dx.doi.org/10.1093/aob/mci262>.

- Gray, J. C., Sullivan, J. A., Hibberd, J. M., and Hansen, M. R., 2001: Stromules: mobile protrusions and interconnections between plastids. *Plant Biology*, 3: 223–233.
- Hanson, M. R., and Sattarzadeh, A., 2008: Dynamic morphology of plastids and stromules in angiosperm plants. *Plant, Cell and Environment*, 31: 646–657.
- Hanson, M. R., and Sattarzadeh, A., 2011: Stromules: recent insights into a long neglected feature of plastid morphology and function. *Plant Physiology*, 155: 1486–1492.
- Holá, D., Kutík J., Kočová, M., and Rothová, O., 2008: Low-temperature induced changes in the ultrastructure of maize mesophyll chloroplasts strongly depend on the chilling pattern/intensity and considerably differ among inbred and hybrid genotypes. *Photosynthetica*, 46(3): 329–338.
- Holzinger, A., Wasteneys, G. O., and Lütz, C., 2007: Investigating cytoskeletal function in chloroplast protrusion formation in the Arctic-Alpine plant *Oxyria digyna*. *Plant Biology* 9: 400–410.
- Ishikawa, H. A., 1996: Ultrastructural features of chilling injury: injured cells and the early events during chilling of suspension-cultured mung bean cells. *American Journal of Botany*, 83: 825–835.
- Kołodziejek, I., Wał za, M., and Mostowska, A., 2006: Morphological, histological and ultrastructural indicators of maize and barley leaf senescence. *Biologia Plantarum*, 50(4): 565–573.
- Körner, C., and Larcher, W., 1988: Plant life cold climates. In Long, S. P., and Woodward, F. Y. (eds.), *Plants and Temperature*. London: Cambridge, Symposium Society Experimental Biology, Vol. 42, pp. 25–27.
- Kratsch, H. A., and Wise, R. R., 2000: The ultrastructure of chilling stress. *Plant, Cell and Environment*, 23: 337–350.
- Larcher, W., Wagner, J., and Lütz, C., 1997: Effect of heat on photosynthesis, dark respiration and cellular ultrastructure of Arctic-alpine psychrophyte *Ranunculus glacialis*. *Photosynthetica*, 34: 219–232.
- Leddet, P. C., and Geneves, L., 1982: Effects of low temperatures (+6 degrees C and +2 degrees C) on the fine structure of mitochondria and chloroplasts in the *Ephedra* tissues, cultivated *in vitro*. *Annales Des Sciences Naturelles*, 13: 27–49.
- Lewis-Smith, R. I., 2003: The enigma of *Colobanthus quitensis* and *Deschampsia antarctica* in Antarctica. In Huickes, A. H. I., Gieskes, W. W. C., Schorno, R. L. M., van der Vies, S. M., and Volf, W. I. (eds.), *Antarctic Biology in Global Context*. Leiden, The Netherlands: Backham Publishers, pp. 234–239.
- Logan, D. C., 2006: Plant mitochondrial dynamics. *Biochemica et Biophysica Acta*, 1763: 430–441.
- Logan, D. C., and Leaver, C. J., 2000: Mitochondria-targeted GFP highlights the heterogeneity of mitochondrial shape, size and movement within living plant cells. *Journal of Experimental Botany*, 51(346): 865–871.
- Lütz, C., 1987: Cytology of high alpine plants. Microbody activity in leaves of *Ranunculus glacialis*. L. *Cytologia*, 52: 679–686.
- Lütz, C., 2010: Cell physiology of plants growing in cold environments. *Protoplasma*, 244: 53–73.
- Lütz, C., and Engel, L., 2007: Changes in chloroplast ultrastructure in some high-alpine plants: adaptation to metabolic demands and climate? *Protoplasma*, 231: 183–192.
- Lütz, C., Bergweiler, P., Di Piazza, L., and Holzinger, A., 2012: Cell organelle structure and function in alpine and polar plants are influenced by growth conditions and climate. In Lütz, C. (ed.), *Plants in Alpine Regions: Cell Physiology of Adaptation and Survival Strategies*. Vienna: Springer-Verlag.
- Mostowska, A., 1999: Response of chloroplast structure to photodynamic herbicides and high oxygen. *Z Naturforsch*, 54c: 621–628.
- Nessler, C. L., and Wernsman, E. A., 1980: Ultrastructural observations of extranuclear temperature-sensitive lethality in *Nicotiana tabacum* L. *Botanical Gazette*, 141: 9–14.
- Parnikoza, I., Kozeretska, I., and Kunakh, V., 2011: Vascular plants of the maritime Antarctic: origin and adaptation. *American Journal of Plant Sciences*, 2: 381–395.
- Pennell, R. I., and Lamb, C., 1997: Programmed cell death. *The Plant Cell*, 9: 1157–1168.
- Romero, M., Casanova, A., Iturra, G., and Reyes, A., Montenegro, G., and 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.
- Schattat, M., Barton, K., Baudisch, B., Klösgen, R. B., and Mathur, J., 2011: Plastid stromule branching coincides with contiguous endoplasmic reticulum dynamics. *Plant Physiology*, 155: 1667–1677.
- Shaw, D. J., and Gray, J. C., 2011: Visualisation of stromules in transgenic wheat expressing a plastid-targeted yellow fluorescent protein. *Planta*, 233: 961–970.
- Stefanowska, M., Kura, M., and Kacperska, A., 2002: Low temperature-induced modifications in cell ultrastructure and localization of phenolics in winter oilseed rape (*Brassica napus* L. var. *oleifera* L.) leaves. *Annals of Botany*, 90: 637–645.
- Yen, C. H., and Yang, C. H., 1998: Evidence for programmed cell death during leaf senescence in plants. *Plant Cell Physiology*, 39: 922–927.
- Zamora, P., Rasmussen, S., Pardo, A., Prieto, H., and Zúñiga, G. E., 2010: Antioxidant responses of *in vitro* shoots of *Deschampsia antarctica* to polyethylene glycol treatment. *Antarctic Science*, 22: 163–169.

MS accepted October 2014