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Structure of *Deschampsia antarctica* Desv. anther and pollen grain under the confocal microscope

Budowa pylnika i ziarna pyłku *Deschampsia antarctica* Desv. w mikroskopie konfokalnym

ABSTRACT

The structure of the anther and pollen grain was investigated in an Antarctic plant *Deschampsia antarctica* Desv. under a confocal microscope (CLSM). The Antarctic hair grass is one of the two native vascular plants growing in Antarctica. The structure of *D. antarctica* stamens with their short filaments and elongated anthers is typical of the family *Poaceae*. Microsporogenesis and development of *D. antarctica* pollen grains proceeds in a way typical of angiosperms from the family *Poaceae*. Beside the pistil, the hermaphroditic flower has three stamens with numerous pollen grains in pollen loculi. The monoporate and heteropolar pollen grains have a porus located at the distal pole. When observed under the confocal microscope (CLSM), *D. antarctica* microspores and pollen grains packed tightly inside the microsporangium exhibit strong fluorescence after eosin staining (green fluorescence). The use of calcofluor yielded blue fluorescence of anther endothecial cell walls. The *D. antarctica* anther endothecium is formed of a single layer of cells, although more than one layer of cells were observed at some sites.

Keywords: Antarctica, *Deschampsia antarctica*, pollen structure, anther

STRESZCZENIE

Budowę pylnika i ziaren pyłku badano za pomocą mikroskopu konfokalnego (CLSM) u antarktycznej rośliny *Deschampsia antarctica* Desv. Śmiałek antarktyczny jest jedną z dwóch rodzimych roślin naczyniowych rosnących na Antarktydzie. Pręciki *D. antarctica* mają budowę typową

dla rodziny *Poaceae* z krótką nitką i wydłużonymi pylnikami. Mikrosporogeneza i rozwój ziaren pyłku *D. antarctica* przebiega w sposób typowy dla roślin okrytozalążkowych z rodziny *Poaceae*. W hermafrodytycznym kwiecie obok słupka występują trzy pręciki, z licznymi ziarnami pyłku w komorach pyłkowych. Ziarna pyłku są jednoporowe i różnobiegunowe, z porusem położonym na biegunie dystalnym. Mikrospory i ziarna pyłku u *D. antarctica* ułożone ściśle wewnątrz mikrosporangium, obserwowane w mikroskopie konfokalnym (CLSM) wykazują silną fluorescencję po zabarwieniu eozyną (zielona fluorescencja). Po zastosowaniu kalkafluoru ściany komórek endotecjum pylnika fluoryzują na niebiesko. Endotecjum pylnika *D. antarctica* zbudowane jest z jednej warstwy komórek; w niektórych miejscach obserwowano więcej niż jedną warstwę komórek.

Słowa kluczowe: Antarktyka, *Deschampsia antarctica*, struktura pyłku, pylnik

INTRODUCTION

Modern flora of Antarctica is represented by two species of vascular plants: *Colobanthus quitensis* (Kunth) Bartl. and *Deschampsia antarctica* Desv. (13). *D. antarctica* (Antarctic hair grass) has diverse morphology and anatomy. The diversity is primarily associated with the place of occurrence. As indicated by the hitherto studies, *D. antarctica* plants grow in wet, fertile habitats 30–40 m from the coastline and reach a height of 6–7cm; they have 5–8 leaves with flat, spreading, 3–7 ribbed leaf blades (9). Moreover, plants occurring in such habitats remain green longer than plants growing in dry and poor localities, in which they reach a height of merely 2–3 cm and have folded leaves hidden in the leaf sheath (7). *D. antarctica* produces very small, bisexual, morphologically diverse flowers forming 2–4 spikelets. Typical open (chasmogamous) flowers with 40–52 pollen grains in the sporangium were observed in *D. antarctica* in the Antarctic summer, whereas the so-called cleistogamous closed flowers developing at the end of the vegetation season had anthers filled with a small amount of pollen grains reaching from 20 to 30 (8).

In the natural environment, *D. antarctica* exhibits unusual tolerance to adverse and changeable conditions. The Antarctic hair grass is a model plant for investigations of plant adaptation to extremely harsh and highly changeable environmental conditions (9). The exceptionally inhospitable environment of Antarctica is related to abiotic factors, i.e. low temperature, the extremely short vegetation season (1), rapid temperature changes, cyclic freeze-thaw and wet-dry periods, strong cyclonal winds (3), long days and intense light radiation (including UV-B) in summer (and shortage of sunlight in winter), salinity, and high habitat variability (1, 3). These particularly severe living conditions offered by polar deserts constitute an object of ecologists' investigations into the possibility of plant growth and development in such extreme environments (6). In their reports on plant growth in Arctic conditions, authors distinguish 4 types of adaptive strategies in plant lives, which are associated with specific morphological forms, i.e. miniaturization, oligomerization, compensation, and geophytization (4). Besides these examples of life strategies, vascular plants growing in Antarctica have evolved a variety of specific adaptive, anatomical, and physiological traits. The *D. antarctica* species examined herein persists in this habitat and colonizes new areas that appear due to glacial melting thanks to the specific features that the species has evolved. In the area of the Polish Antarctic Station, there are places where *D. antarctica* tufts die due to the persistent snow and snow-ice cover. In the same vegetation season during the second half of summer, massive appearance of seedlings is observed, which emerge from seeds that remain in flower involucres and stay together with inflorescences on dead parental plants (6). Leaves produced by *D. antarctica* exhibit a highly xerophytic character. *D. antarctica* leaf blades have a small surface area and are V-shape folded, which provides a cover for stomatal apparatuses on the adaxial surface, thereby ensuring a low transpiration level (9).

A majority of published investigations concern the physiological and ecological aspects of

D. antarctica. Considerably fewer publications are focused on the structure of the anther and pollen grains in this plant (17). The author examined pollen grains from the Antarctic hair grass by scanning microscope (SEM). In turn, in this study, we concentrated on the structure of the anther and pollen grains revealed by confocal microscopy (CLSM).

MATERIAL AND METHODS

Deschampsia antarctica, a vascular plant originating from the Antarctic geobotanical zone was the object of the study. Flower buds and flowers of *D. antarctica*, a plant from the family *Poaceae*, were collected near the H. Arctowski Polish Antarctic Station. The plant material was fixed in a 3:1 mixture of ethanol and acetic acid.

Anthers were isolated from the collected material for detailed observation of pollen grains under a confocal microscope. Prior to the observations under the confocal microscope, the material was washed three times in water and stained with eosin Y (1g /100 ml distilled water). Eosin Y is an acidic dye used for staining basic (acidophilic) molecules of cellular structures at the 514 nm wavelength.

For detailed observation of the cell walls of the pollen grains, anthers with microspores were additionally stained with a mixture of 0.5% and 1% calcofluor in a KOH solution. After a 30-min incubation, the anthers were rinsed three times in water and observed under the confocal microscope (CLSM) at the 405 wavelength.

RESULTS AND DISCUSSION

In the natural environment, *D. antarctica* mainly employs vegetative propagation, although it produces seeds as well. The plant blooms abundantly and the flowers appear nearly every year. The species forms chasmogamous and cleistogamous flowers. In Antarctica, *D. antarctica* localities are found on islands, e.g. King George Island and along the western coast of the Antarctic Peninsula (Fig. 1). Under the confocal microscope, eosin-stained *D. antarctica* anthers exhibited a morphological structure typical of the family *Poaceae* and varied fluorescence. Pollen grains and filaments were particularly strongly fluorescent. *D. antarctica* anthers had a slim and elongated shape (Fig. 2). Anthers have pollen loculi, in which pollen grains develop from the archespore. In the observed anther, pollen grains were tightly packed inside the sporangium. They exhibited different sizes, had an irregular shape, and emitted distinct fluorescence (Figs. 2, 3). Mature *D. antarctica* pollen grains are trinuclear, and their sporoderm has one aperture (porus), which becomes visible at magnification (Fig. 4).

Crushed calcofluor-stained fragments of the *D. antarctica* microsporangium containing pollen grains exhibited strong fluorescence visible under the confocal microscope. The blue fluorescence of endothecial cell fragments displayed irregular thickening of cell walls. The endothecial cells were irregularly shaped and their thickenings were more pronounced than those in other plants. Pollen grains squeezed out of the anthers and observed at magnification after eosin Y-staining showed two distinct nuclei. Cell organelles emitted fluorescence much

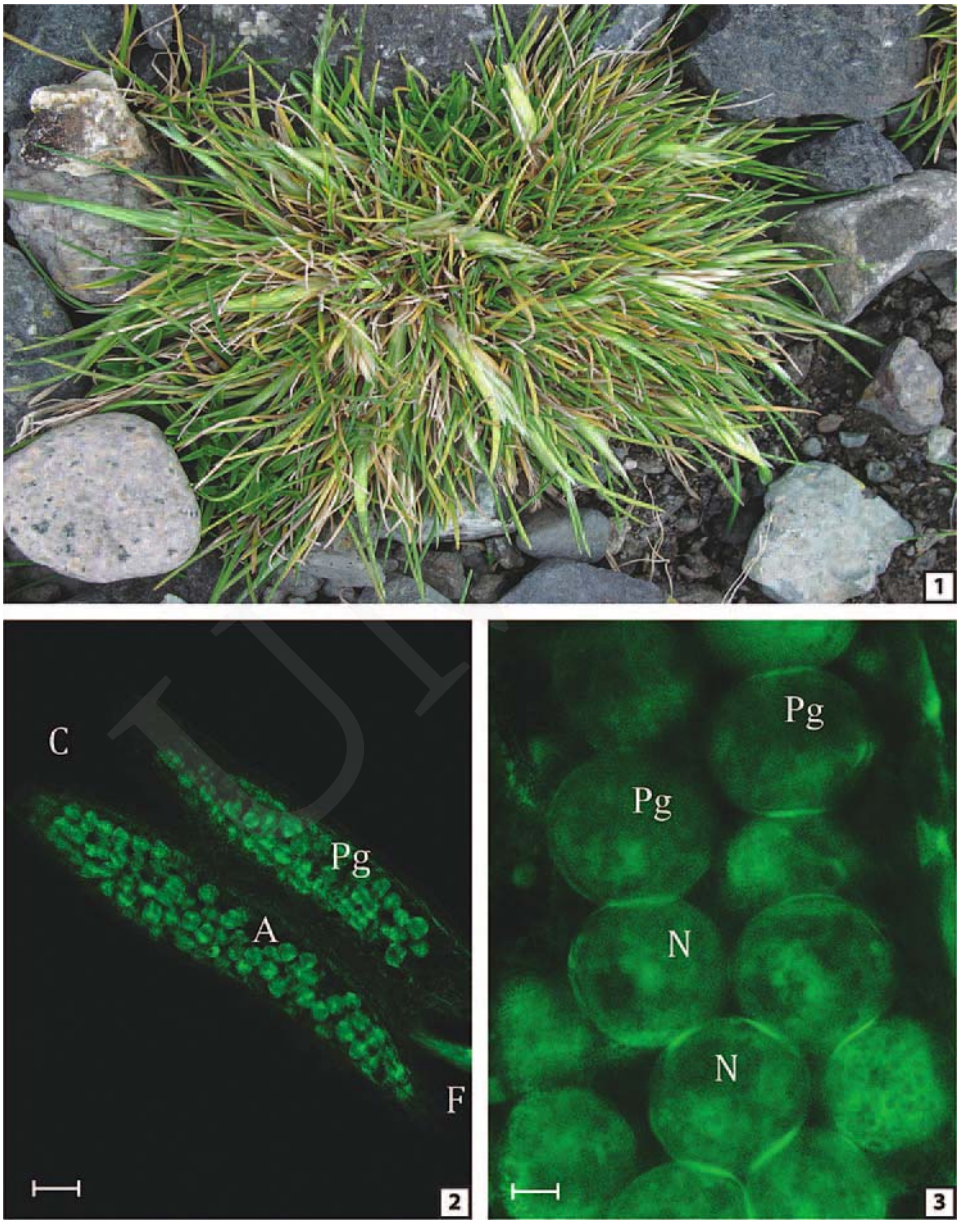

Fig. 1. Morphology of a *D. antarctica* grass tuft during Antarctic summer (2010)

Fig. 2. Morphological structure of the *D. antarctica* anther. Visible fluorescence of eosin Y-stained pollen grains in the anther under the confocal microscope (CLSM). Scale = 65 µm, F – filament, C – anther head, Pg – pollen grain, A – anther

Fig. 3. *D. antarctica* pollen grains inside the microsporangium. Eosin Y-stained preparation under the confocal microscope (CLSM). Scale = 7 µm, N – nucleus, Pg – pollen grain

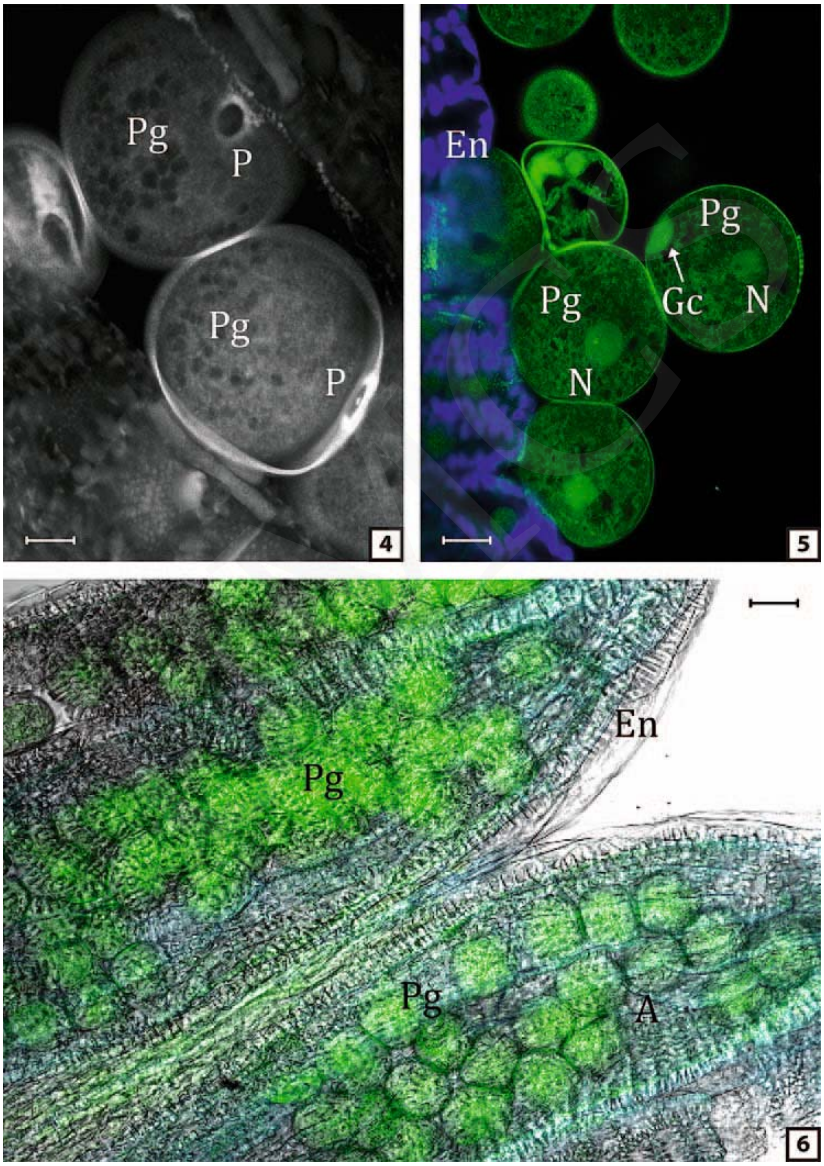

Fig. 4. Sections of eosin Y-stained pollen grains inside the anther with pori visible under the confocal microscope (CLSM). Scale = 5 µm. P – porus, Pg – pollen grain

Fig. 5. Pollen grains squeezed out of the anthers. Green fluorescence of eosin Y-stained pollen grains; after calcofluor staining, the endothecium walls exhibit blue fluorescence. Scale = 7 µm. N – nucleus, En – endothecium, Pg – pollen grain, Gc – generative cell

Fig. 6. Fragments of two anthers with numerous, tightly packed pollen grains emitting green fluorescence after eosin Y staining and blue fluorescence of the endothecium walls after calcofluor staining; a confocal microscope view (CLSM). Scale = 32 µm. Pg – pollen grain, En – endothecium, A – anther

more strongly than the surrounding cytoplasm (Fig. 5). Tightly packed pollen grains were visible in eosin Y-stained anthers. The anthers were surrounded by an endothecium layer (Fig. 6). Additionally, the fibrous structure of cell walls in the anther endothecium and strongly fluorescent pollen grains inside the microsporangia were visible. The anther wall is composed of a layer of elongated endothecial cells exhibiting distinct fluorescence. They form long bands, which contribute to the characteristic fibrous structure of the anther (Figs. 5, 6).

In the *D. antarctica* grass species investigated, the pollen grain size ranged from 25 μm to 35 μm, i.e. they are medium-sized; the size of grass pollen grains is usually in the range from 16 μm to 50 μm in wheat, and 75–100 μm in maize (15). They contain a vegetative cell and a lenticular generative cell pressed against the pollen grain wall (Fig. 5), the pollen grain on the right), which is divided during male gametophyte development into two sperm cells reaching the embryo sac through the pollen tube. Grass pollen grains have spherical, oval, or ellipsoid shape. Its sporoderm has pores (pori), through which the pollen tube can germinate. The pori in the grass species examined are usually roundish, monoporate, and heteropolar; they are surrounded by a convex annulus and covered with the exine operculum. Gelatinized and swollen pectin disc located below the aperture disrupt the sporopollenin membrane, to which the operculum is attached, resulting in formation of an opening for pollen tube germination.

The mode of microsporogenesis and male gametogenesis (not shown in figures in this paper) in *D. antarctica* was typical of angiosperms. The pollen grains of the Antarctic hair grass are tightly packed inside the anther; the different sections of the anther show diverse density and irregular shapes of the pollen grains contained in the anther.

The flower of the grass species studied is composed of the pistil formed by the ovary and two plumose branched stigmata, which capture pollen from the air (12). Below the pistil, there are three usually pendulous stamens on relatively long filaments with yellowish or reddish anthers. Each anther head consists of two anther halves with two pollen sacs in each.

Similar to other grasses, *D. antarctica* anthers are composed of four loculi with well-developed multi-layered walls typical of most *Angiospermae* (11). In the anther head, there are four paired microsporangia separated by connective tissue containing a vascular bundle (14). The anther wall differentiates centripetally, following the pattern characteristic of monocotyledonous plants. In the anther maturity stage, it is composed of epidermis, a fibrous layer (endothecium) with characteristic fibrous wall thickenings expanding from the inner cell towards the epidermis, a single or double degenerating middle layer, and a binuclear or, less frequently, mononuclear anther tapetum (2). Usually, the tapetum is a single, dense, nutrient-rich layer of cells surrounding sporogeneous cells (16).

A characteristic feature of *Poaceae* is the single-layered secretory tapetum

with binuclear cells that initially surround microsporocytes arranged in a single layer, and a single layer of tetrads in the successive stages of anther development. Fibrous thickenings that ensure opening of a mature anther are formed in the endothecium layer. The centre of the anther loculus is filled with sporogenous tissue, in which microsporocytes are connected by plasmodesmata until some stage (2). The pollen sac is filled with archesporial tissue, in which the number of archesporial cells increases through mitotic divisions until meiosis begins and the archesporial cells develop into microsporocytes. Microsporocytes undergo meiosis, during which they become isolated from each other with thick callose walls.

The process of microsporogenesis in *D. antarctica* is accomplished by successive cytokinesis. A tetrad of haploid cells developing into microspores is formed inside the callose wall of each microsporocyte. During microspore formation, the common callose wall surrounding the individual tetrads disappears gradually. The tapetal tissue with its nutritious substances disintegrates and disappears as well. The enlarged microspores are surrounded by double walls: a thin and delicate intine wall composed of cellulose-pectin substances and a thick and resistant exine wall containing sporopollenin (10).

After release from the previous microsporocyte wall, mononuclear *D. antarctica* microspores undergo two mitotic divisions in the process of formation of a male gametophyte and sperm cells, i.e. microgametogenesis. After the first mitosis, a vegetative cell and a lenticular generative cell pressed against the pollen grain wall are formed (14). After moving away from the wall, the generative cell surrounded by the cytoplasmic membrane of the sister vegetative cell undergoes the second mitotic division, whereupon two sperm cells are formed with nuclei surrounded by a thin cytoplasm layer (5). Sperm cells located inside the pollen grain have a spindle-like shape or they are rounded at one end and pointed at the other (10).

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