High anatomical and low genetic diversity in *Deschampsia antarctica* Desv. from King George Island, the Antarctic

Katarzyna J. CHWEDORZEWSKA1, Irena GIEŁWANOWSKA1,2, Ewa SZCZUKA3 and Anna BOCHENEK2

1 Zakład Biologii Antarktyki, Polska Akademia Nauk, Ustrzycka 10/12, 02-141 Warszawa, Poland <kchwedorzewska@o2.pl>

2 Katedra Fizjologii i Biotechnologii Roślin, Uniwersytet Warmińsko-Mazurski, Oczapowskiego 1A, 10-719 Olsztyn, Poland <i.gielwanowska@uwm.edu.pl>

3 Zakład Anatomii i Cytologii Roślin, Uniwersytet Marii Curie-Skłodowskiej, Akademicka 19, 20-033 Lublin, Poland

**Abstract**: Morphological, anatomical, and genetic differences between *Deschampsia antarctica* Desv. (Poaceae) growing in the Antarctic in dry site as well as plants of the same species growing near the seashore were investigated. The plants from the two microhabitats were found to differ morphologically in size and in the arrangement, shape, and color of the leaves. The leaves of plants growing in the dry site had stronger xerophytic features than those growing in the wet site. The anatomical structure of the root of plants growing in wet conditions showed strong reaction to stress factors operating at the seashore. Molecular profiling of *D. antarctica* individuals showed low variability within the analysed populations. The results of the present study conclude that the two different forms of *D. antarctica* are ecotypes.

**Key words**: Antarctic, *Deschampsia antarctica*, morphology, anatomical features, leaf, root, AFLP.

**Introduction**

The Polar Front is the strongest of a series of eastward-flowing jets of the Antarctic Circumpolar Current. This barrier strongly limits the north-south exchange of water and isolates the Southern Ocean and, thus, the whole of Antarctica (Clarke *et al.* 2005). Thermal and biogeographical isolation are the main reasons why ecosystems in the Antarctic regions are relatively simple compared to lower latitudes (Bokhorst *et al.* 2007). Only *Deschampsia antarctica* Desv. (Antarctic hairgrass, Poaceae), a representative of a widespread genus, which is also well represented in the Southern Hemisphere, and *Colobanthus quitensis* Bartl. (Antarctic pearlwort, Caryophyllaceae), belonging to an old Antarctic genus with a center...
on New Zealand, successfully colonized maritime Antarctic. Several hundred vascular plant species occur in Tierra del Fuego but only two above mentioned species have successfully crossed the Drake Passage and colonized maritime Antarctic. *D. antarctica* and *C. quitensis* occur extensively from South Orkney and South Shetland Islands in the north, to the Tierra Firma Islands and the eastern side of the south-western Antarctic Peninsula. Outside the Antarctic biome they occur on the sub-Antarctic islands, and are common in the Falkland Islands and Tierra del Fuego. *D. antarctica* reaches central Chile and Argentina (Moore *et al.* 1965). *D. antarctica* was first described from the Antarctic at the South Orkney Islands in 1823 (Edwards and Greene 1973).

*Deschampsia antarctica* is one of the main components of the Antarctic herb tundra formation (Longton 1988). Its distribution depends on the nutrient supply in the soil, on water conditions in the area, and presence of animal colonies (Nędzarek and Chwedorzewska 2004). It grows abundantly in the vicinity of penguin rookeries and gathering places of large mammals, sometimes forming continuous patches of large or small clusters, revealing good tolerance to direct contact with animal feces. In favorable sites *Deschampsia* forms extensive closed communities covering several hundred square meters. Plants in such habitats are green, robust, have well-developed foliage and erect culms. At the most elevated localities far from animal colonies the grass occurs as individual tufts scattered over a fairly wide area and is mainly restricted to cracks between the rocks. *D. antarctica* inhabits even restricted areas of flat ground in front of high sea cliffs. The plants there are much smaller, olive-green in colour and form small, single, flat tufts. In some places the grasses form clusters together with cushions of *Colobanthus quitensis* or communities of mosses or lichens.

*D. antarctica* demonstrated high adaptational plasticity to different soil conditions and high resistance to overmanuring, as well as ability to survive in soils practically lacking nutrient (Smith 2003; Krywult *et al.* 2003; Nędzarek and Chwedorzewska 2004). Analysis of the water flow in plants growing in the costal area demonstrated that nitrogen and phosphorous ions were present at very high concentrations. In plants growing far from animal colonies, in areas where the landscape resembles a polar desert with freshly weathered rock, very mineral poor...
Rh – root hairs. Arrows point transverse sections of fungal hyphae. Scale bar is 50 μm. g. Transverse section of root of D. antarctica growing in the wet habitat. The vascular bundles (Vb), pericycle (P), and endodermis (En) surrounded by irregular cortex (Ko) cells. Arrows point transverse sections of fungal hyphae. Note short root hairs (Rh) with osmophilic material in the walls. Scale bar is 50 μm.
soil and glacial deposits almost devoid of vegetation, the concentration of ions was several magnitudes lower (Nędzarek and Chwedorzewska 2004).

Physiological and biochemical processes are connected with the anatomical structure of plant organs such as leaves and roots. Extensive variations of the leaf surface and anatomy of *D. antarctica* was reported by Romero *et al.* (1999). Using light and scanning microscopes, the authors observed differences between plants growing in the natural, Antarctic conditions and in their laboratory clones. Some observations concerning the leaf anatomy of Antarctic hairgrass were reported by Giełwanowska and Szczuka (2005) and Giełwanowska *et al.* (2005). However, in both papers the authors paid attention mainly to the ultrastructure of organelles in the leaf mesophyll cells of *D. antarctica*. In this study, on the other hand, we focus on the morphology and anatomy of the leaf and the root of *D. antarctica* growing in two different Antarctic microhabitats. The aim of the genetic part of the study was to compare variation revealed by an anatomic study with that of AFLP (Amplified Fragment Length Polymorphism; Vos *et al.* 1995) fingerprints. The hypothesis is that plants exhibiting anatomical differences are also similar at the DNA level.

Material and methods

**Plant sampling.** — Individuals of *Deschampsia antarctica* were collected from maritime Antarctica, on the western shore of Admiralty Bay (King George Island, the South Shetland Islands) in the vicinity of the Henryk Arctowski Polish Antarctic Station (62°09′S, 58°27′W) in the area of ASPA 128. The investigated samples originated from plants (Fig. 1) growing in two different microhabitats. One locality (A) was situated at the hilltop near Puchalski’s grave, approximately 300 m from the seashore; it lies on old ornithogenic soil, but far from present day bird colonies and gathering places of large mammals. It is occasionally manured by flying birds such as skuas or giant petrels and always exposed to very strong drying westerly winds. The site at the hilltop is very dry and *Deschampsia antarctica* grows there as scattered plants accompanied by *Colobanthus quitensis*, mosses and lichens. The plants of *D. antarctica* growing in this habitat are very small and did not exceed 5 cm in height. Their leaves are short and pressed close together. Most of them are olive-green, mixed with some dead brown leaves. They usually form small flat individual tufts.

The second locality (B) was situated along the shore, exposed to seawater aerosols, occasionally flooded by seawater and strongly fertilized by water rich in biogens flowing down from a penguin rookery. The site located along the shore consists of numerous big, dense patches of *D. antarctica*, uninterrupted by other plant species, spread over approximately one square meter. In this wet habitat plants were taller, greener, more robust and having well-developed, long, widely spread foliage.
forming erect tufts. Plants from those two localities were collected at random manner but growing at least 3 m from each other. Five to six replicates of samples were collected from both forms, flat tufts and dense mats. For genetic analyses, whole plants were dried between two pieces of paper, and then stored at room temperature.

**Microscopic analyses.** — For microscopic analyses of *D. antarctica*, leaf and root sections, 2–3 mm in length, five from locality A and five from locality B, were fixed in 3.5% glutaraldehyde in 0.1M phosphate buffer (pH 7.0) for 10h at room temperature. After washing in two changes of 0.1 M phosphate buffer, the samples were postfixed overnight in 2.5% osmium tetroxide in the same buffer. After dehydration in a graded alcohol series, the material was embedded in Poly Bed 812 resin. Semi-thin (1–2 μm thick) sections, stained with 1% solution of toluidine blue in 0.5% water solution of borax were examined with the light microscope.

**DNA extraction.** — DNA was extracted from about 100 mg dry leaf tissue following the manufacturer’s recommendation (Qiagen: DNeasy Plant Mini Handbook for DNA isolation from plant tissue). Purity and quantity of the samples were determined spectrophotometrically. DNA integrity and lack of RNA impurities were tested in 1.4% agarose gels (1x TBE buffer and ethidium bromide (0.5 μg/ml) at 20 V/cm). For routine purposes standard dilutions 10 μg/ml were prepared.

**AFLP analysis.** — The Amplified Fragment Length Polymorphism (AFLP) technique was performed according to the procedure described by Vos et al. (1995)
with minor modification (Chwedorzewska et al. 2002). Briefly, 250 ng of genomic DNA was digested with EcoRI and Msel and ligated to the appropriate adaptors. A pre- (weakly selective) amplification step was performed in the presence of primers with one selective nucleotide ("A" for EcoRI and "C" for Msel). For the selective amplification, primer combinations (E-AAA/M-CAA, E-ACT/M-CTT, E-AAT/M-CTT, E-AAT/M-CCA) with two additional nucleotides at the 3′-ends were used. The EcoRI and Msel compatible primers were labelled at their 5′-ends with gamma – 32P ATP. PCR products were separated on 5% PAGE and exposed to X-ray film at −70°C overnight.

Data analysis. — Visible, reproducible and polymorphic bands were scored as presence (1) and absence (0) and arranged in a matrix for further evaluation. The estimates of similarity were based on Euclidean distance and clustering was performed using unweighted pairgroup with arithmetic averages (UPGMA) clustering method (Sokal and Michener 1958). The results are presented as dendrogram (Fig. 2).

Results

Microscopic observation. — The leaves of D. antarctica plants growing in the dry, exposed habitat are rolled and almost closed (Fig. 1c). The bulliform cells of those leaves were not well developed and did not differ significantly from the neighbouring epidermal cells. The external epidermis consists of cells similar in size and shape which were covered by a cuticular layer thicker than in the internal epidermis. The cells of the internal epidermis differed significantly in size and shape. A transversal section of the internal epidermis showed the presence of stomata. The sclerenchymatic fibers were present at the margins, and very rare strands of them occurred between the ribs on the adaxial surface. The mesophyll, undifferentiated into palisade and spongy layers was composed of large, rather isodiametric chlorenchyma cells with few lacunae. In each of the three ribs, a collateral vascular bundle was visible: in the central rib the vascular bundle was the most prominent.

Transverse sections of the central part (Fig. 1d) and the marginal part (Fig. 1e) of a leaf from a plant growing in the wet habitat show dissimilarity with leaves of D. antarctica growing in the dry habitat described above. The leaves were rather flat or slightly folded and usually consisted of five ribs. Among epidermal cells, the most remarkable were bulliform cells. They were very large and were situated on the adaxial surface at the bases of the furrows, between the ribs. The sclerenchymatic fibers were present at the apex of each rib and between the ribs just beneath the external epidermis. The mesophyll cells were irregular in shape and size and lacunae were very large and numerous.
A transverse section of the root of *D. antarctica* growing in the dry site showed the typical structure of this organ (Fig. 1f). The root epidermis, typically uniseriate, developed root hairs containing osmophilic material in the walls. The cortex was built out of big and rather regularly shaped parenchymatous cells. The cells were arranged in regular ring-shaped layers. The presence of schizogenous intercellular spaces was typical of the root cortex. The innermost cortical layer — endodermis — consists of relatively smaller cells (in comparison to the other cells of the cortex) with U-shaped thickenings confined to the radial and inner tangential walls. The thickenings were very prominent and especially clearly visible after toluidine blue staining. The center of the oligoarchic root was filled with 4 vascular bundles surrounded by one layer of pericycle.

Layers of anatomical structure similar to those described above are present in the transverse section of the root of *D. antarctica* growing in the wet habitat (Fig. 1g). The shape of such a root, however, is irregular, and the cortex cells differed significantly in size and shape. The thickenings of the endodermis were not so prominent as in the plants from the dry site. The long root hairs contained bigger amounts of osmophilic material in the walls.

**AFLP.** — In total, four selective primer combinations amplified 273 bands that were generated from all DNA templates isolated and processed according to the AFLP approach. The number of DNA fragments generated by an individual primer pair varied from 19 to 97 with an average of 68. All primer pairs generated polymorphic signals and their number ranged between 3 and 44. Nearly 35% of the identified DNA fragments exhibited polymorphism. Plants from the dry site exhibited somewhat more polymorphism than those from the shore site (Table 1, Fig. 1).

**Table 1**

<table>
<thead>
<tr>
<th>Primers pair code</th>
<th>Detected fragments (in total)</th>
<th>Polymorphic fragments</th>
<th>% of polymorphism</th>
</tr>
</thead>
<tbody>
<tr>
<td>E-AAA/M-CAA</td>
<td>19</td>
<td>3</td>
<td>16</td>
</tr>
<tr>
<td>E-ACT/M-CTT</td>
<td>97</td>
<td>44</td>
<td>45</td>
</tr>
<tr>
<td>E-AAT/M-CTT</td>
<td>79</td>
<td>23</td>
<td>29</td>
</tr>
<tr>
<td>E-AAT/M-CCA</td>
<td>78</td>
<td>25</td>
<td>32</td>
</tr>
<tr>
<td>Total</td>
<td>273</td>
<td>178</td>
<td>35</td>
</tr>
</tbody>
</table>

**Discussion**

The morphology of *D. antarctica* plants growing in two different Antarctic microhabitats differs considerably. Plants from the dry highland site and from the wet shore site show dissimilarity in size and also in the arrangement, shape and
color of their leaves. Morphological differences dependent on the growing site were observed in other species, e.g., *Saxifraga oppositifolia* (Brysting et al. 1996) and *Saxifraga caespitosa* from Arctic (Chwedorzewska et al. 2005). The leaf blades of *D. antarctica* plants growing in a dry, exposed habitat were strongly folded and V-shaped. At least partially, this feature seems to be connected with exceptionally severe environmental conditions. The significance of such a leaf shape in the Antarctic habitat was extensively discussed by Romero et al. (1999). The V-shaped folded leaves are, among others, due to the small size of the bulliform cells. This was observed also in *Deschampsia flexuosa* growing outside Antarctica (Metcalfe 1960). The large bulliform cells occurring in the leaves of *D. antarctica* from the wet habitat make these organs almost flat or only slightly folded.

Besides the difference in the size of the leaf blade bulliform cells, leaves of Antarctic hairgrass from microhabitats A and B show differences in the thickness of the cuticle covering the abaxial epidermis, the quantity and arrangement of sclerenchymatic fibers, the shape of the mesophyll cells, and the presence of lacunae in the mesophyll. The features of the leaves of plants growing in site A are more xerophytic than those from the wet habitat. Other anatomical, mainly ultrastructural features of leaves of *D. antarctica* growing in three different environmental conditions have been presented by Gielwanowska and Szczuka (2005) and Gielwanowska et al. (2005).

Strong differences were also observed in the anatomical structure of the roots of the examined plant. In a transverse section of the root of *D. antarctica* growing in the dry site, the cells and layers of the root were more regular than in plants growing in the wet habitat. Moreover, the root hairs of plants growing in the wet habitat were longer and contained more osmophilic material than those from the dry habitat. It can be argued that the anatomical features of the root of plants growing in wet conditions reflects strong reaction to the stress factors operating at the seashore (Bravo et al. 2001; Levitt 1980).

Some genetic differences detected by AFLP analyses probably exhibited intrapopulation variability characteristic for *Deschampsia antarctica* (Chwedorzewska et al. 2004). The clustering analysis shows that both populations are genetically very similar. Dispersal of seeds and even the whole plants by westerly winds or by birds is possible from the elevated site down to the beach site. Moreover, the polymorphism exhibited by plants from the dry site was larger than that exhibited by plants from the beach site, probably because the plants from the latter site, growing as big continuous patches, reproduce mainly vegetatively. Seeds of *D. antarctica* in this wet and fertile site are produced very seldom (Gielwanowska 2005).

The study showed that observed morphological differences are only due to environmental differences. Also the present research shows that variation detected at the anatomical level reflects only phenotypic plasticity of the species. It is concordant with results obtained for other species, inhabiting similar harsh environments, but situated at the opposite side of the globe, in the Arctic. Strongly different phe-
notypes of *Saxifraga caespitosa* growing at the same sites did not show any genetical differences. But in that case the morphological variation could not be explained by environmental factors, like water availability, nutrient supply and light exposure (Chwedorzewska et al. 2004). Our results, as well as other previous studies, can not validate the hypothesis that plants inhabiting harsh conditions show unusually high phenotypic plasticity. The data clearly show the insufficiency of morphological observations, which are still the base of many studies.

**Acknowledgements.** — This research was supported by grant number IPY/26/2007.

**References**


Received 12 May 2008
Accepted 5 November 2008