Revista Chilena de Historia Natural
72: 411-425, 1999

Leaf anatomy of *Deschampsia antarctica* (Poaceae) from the Maritime Antarctic and its plastic response to changes in the growth conditions

Anatomía foliar de *Deschampsia antarctica* (Poaceae) de la Antártida Marítima y su respuesta plástica a variaciones de las condiciones de crecimiento

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ABSTRACT

The leaf blade anatomical features of *Deschampsia antarctica Desv.* growing in Robert Island, South Shetland Islands, Maritime Antarctic (62°22'S 59°43'W) and in clones cultivated in the laboratory for two years, at 2 ± 1.5 and 13 ± 1.5 °C and 180 μmol $m^{-2} s^{-1}$ of irradiance were studied by light and scanning electron microcospy. Since *D.antarctica* is growing under the harsh environmental conditions of the Maritime Antarctic for at least five millennia, it is postulated, that their leaf anatomy may show genotypic adaptations to this environment, which should be maintained when clones of this plant are cultivated under different conditions. In this Antarctic habitat, mean air temperature of January was ca. 2.8 °C (< 8 to –2.5 °C) and the maximal irradiance was ca. 2000 mmol $m^{-2} s^{-1}$. A strong variation was found in the anatomical characteristics of the leaf surface and in the leaf cross section, between plants growing in the field and their clones growing at the highest temperature in the laboratory (13 °C). The leaf surface of the Antarctic samples showed more xerophytic characteristics (smaller leaf surface and epidermal cells, higher leaf thickness, higher stomata density and number of cells per area) than the leaves of plants cultivated at 13 °C. Additionally, Antarctic samples presented stomata in both surfaces and epidermal cells with turgid papillae. Therefore, the taxonomic value of epidermal characteristics for identification of Poaceae could be questioned. In the leaf transverse section the vascular bundles of the Antarctic samples appeared surrounded with two bundle sheaths: an outer, with parenchymatous cells without chloroplasts, and an inner or mestome with thick walls. The outer bundle sheath was absent in leafs of plants growing at 13 °C. Xylem of leaf Antarctic samples did not present lacuna and their vessel lumens were smaller than at 13 °C. Leaf anatomical characteristics of plants growing at 2 °C correspond to an intermediate state between the two mentioned conditions. The results suggest that the leaf anatomical features of *D. antarctica* do not correspond to a genotypic adaptation to the harsh environmental Antarctic conditions, but rather to a plastic response of the phenotype to ameliorated growth conditions in the laboratory.

Key words: *Deschampsia antarctica*, Poaceae, Antarctic, leaf anatomy, phenotypical anatomical changes and temperature.

RESUMEN

Se estudia las características anatómicas de la lámina foliar en plantas de *Deschampsia antarctica Desv.* creciendo en Isla Robert, Islas Shetland del Sur, Antártida Marítima (62°22'S 59°43'W) y en clones cultivados en el laboratorio a 2 ± 1,5 °C y 13 ± 1,5 °C y 180 mmol $m^{-2} s^{-1}$ de irradiancia, utilizando microscopía óptica y de barrido. Ya que *D. antarctica* crece, desde aproximadamente cinco milenios, bajo las condiciones ambientales desfavorables de la Antártida Marítima, se postula que su anatomía foliar presentaría adaptaciones genotípicas a este ambiente, las que deberían mantenerse cuando clones de esta planta son cultivados bajo diferentes condiciones. En el hábitat antártico, las temperaturas promedio de enero fueron de aproximadamente 2,8 °C (< 8 a –2,5 °C) y la irradiancia máxima de 2000 μmol $m^{-2} s^{-1}$. Muestras del hábitat y de los cultivos en el laboratorio (13 °C) presentaron una marcada variación en las características anatómicas, de la superficie foliar y del corte transversal de la hoja, con el incremento de la temperatura. La superficie foliar de las plantas creciendo en la Antártida evidenció características más xerofíticas (menor superficie foliar y tamaño de las células epidérmicas, mayor grosor foliar y densidad de estomas y número de células por área) que las hojas de plantas cultivadas a 13 °C. Además, las muestras de la Antártida presentaron estomas en ambas superficies y células epidérmicas con papilas turgentes. En cortes transversales de las hojas, los haces vasculares de las muestras antárticas aparecen rodeados de dos vainas vasculares: una externa, con células parenquimatosas sin cloroplastos y una interna o mestoma con paredes engrosadas. La vaina externa está ausente en las hojas de plantas creciendo a 13 °C. El xilema de las muestras foliares de la Antártida no presentó lagunas, y el lumen de sus vasos fue

(Received 29 November, 1998, accepted 28 May, 1999; managed by L. Corcuera)

menor que a 13 °C. Las características anatómicas foliares de las plantas creciendo a 2 °C ocuparon un lugar intermedio entre las dos condiciones de crecimiento mencionadas. Los resultados sugieren que las características anatómicas foliares de *D. antarctica* no corresponderían a una adaptación genotípica al clima desfavorable de la Antártida, sino más bien a una respuesta plástica del fenotipo a las condiciones de crecimiento menos adversas del laboratorio.

Palabras clave: *Deschampsia antarctica*, Poaceae, Antártida, cambios fenotípicos de la anatomía foliar y temperatura.

INTRODUCTION

Controversies exist on the relationships between the anatomical and morphological characteristics of plants and the environmental conditions (Palta & Li 1979, Levitt 1980). Small cell sizes, thick cell walls, high leaf thickness, and high stomatal density of leaves, among others features, are associated with extreme environmental conditions, such as low temperature (Palta & Li 1979, Körner & Larcher 1988). In tuber-bearing *Solanum* species, Palta & Li (1979) reported that the number and thickness of palisade parenchyma layers and the stomatal index on the upper leaf surface were directly related to their cold resistance and the low temperatures of their habitats. Thus, the anatomical leaf structure of cold resistant species results from their constitutive adaptation to low temperatures, as a consequence of a selection pressure (Tieszen & Helgager 1968, Palta & Li 1979).

In this paper, we will study the anatomical leaf blade characteristics of *Deschampsia antarctica* Desv. (Poaceae), one of two unique native angiosperms that inhabits the Antarctic regions, one of the harshest ecosystems of the world (Edwards & Lewis-Smith 1988, Zúñiga et al. 1996). Since *D. antarctica* has been present in the Maritime Antarctic for at least five millennia (Birkenmeyer et al. 1985, Lewis-Smith 1994), we hypothesized that in the course of the evolution the harsh environmental conditions of the Antarctic have determined the anatomical leaf adaptations that are maintained, when clones of this plant species are cultivated under different conditions.

MATERIALS AND METHODS

Sampling and collection site

The study area was located in Robert Island (South Shetland Islands, Maritime Antarctic 62° 22'S 59° 43'W), where *D. antarctica* grows as isolated small tussocks less than

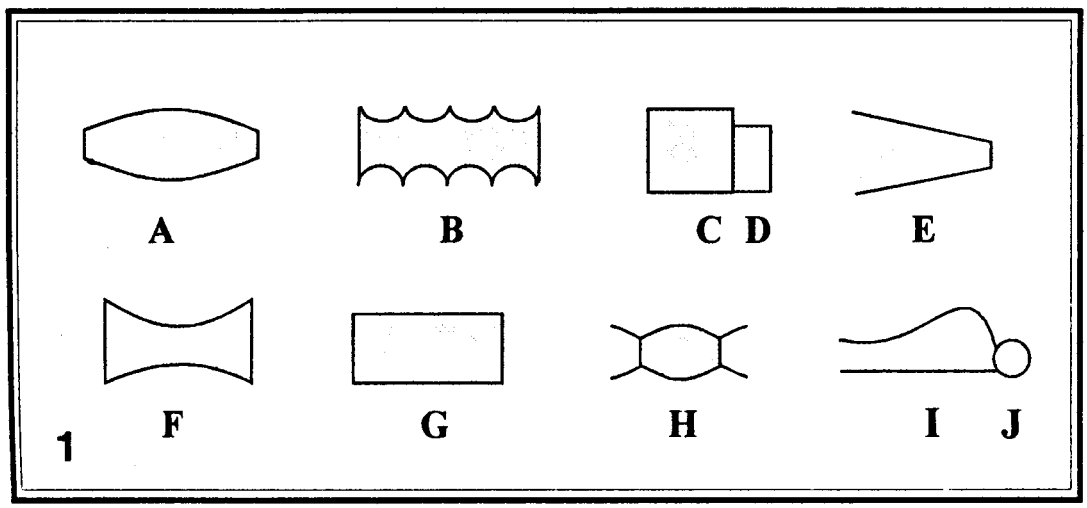


Fig. 1: Schematic representation of the epidermal cell types observed by light microscopy (LM) in the foliar peel of *D. antarctica*. Ten different cell types are shown, designed with the letters A to J.

Representación esquemática de los tipos de células epidérmicas observados por microscopía óptica (MO) en epidermis foliar de *D. antarctica*. Se muestran diez diferentes tipos de células, designadas con las letras A a J.

10 cm high (Casaretto et al. 1994). In this place, the mean air temperature of January was 2.8 °C, being the maximal always below 8 °C and the minimum around -2.5 °C (Zúñiga et al. 1996). The maximum photosynthetic active radiation was approximately 2000 μmol $m^{-2}s^{-1}$ on clear days. More information about the micróclimatic conditions of the Maritime Antarctic habitats are reported by Edwards & Lewis-Smith (1988) and Casanova (1997).

Thirty plants of the Poaceae *D. antarctica* were randomly collected from tussocks in the above mentioned site in January 1995. Since these plants have vegetative reproduction (Casaretto et al. 1994) they can be considered as clones. Leaves from the second node of ten different plants were collected and fixed in FAA (Sass 1958) for anatomical studies. Fresh plants were placed in plastic bags and transported by air to the laboratory in Valdivia (Chile). These plants were grown for two years in climatic chambers under two different temperatures: a) 2 ± 1.5 °C D/N (similar to the mean temperature of the Antarctic summer), and b) 13 ± 1.5 °C D/N (optimal photosynthetic temperature for this species; Edwards & Lewis-Smith 1988). The photoperiod was 16 h, and light intensities ca. 180 μmol $m^{-2}s^{-1}$; the substrate was a mixture of organic soil and turf (3:2 w/w).

TABLE 1

LM measuraments of different epidermal cell types located in the edge, internervic and epinervic zone in leaves of D.antarctica growing in the Antartic and in climatic chamber at 2 ± 1.5 °C and 13 ± 1.5 °C. Dimensions of stomata (guard cells) are also given. Data represent mean of 30 measurements in three leaves of different plants. Standard deviation was lower than 10%. Not present (—), length (L), greater width (w1), smaller width (w^2). Letters represent the cells types us in Fig.1

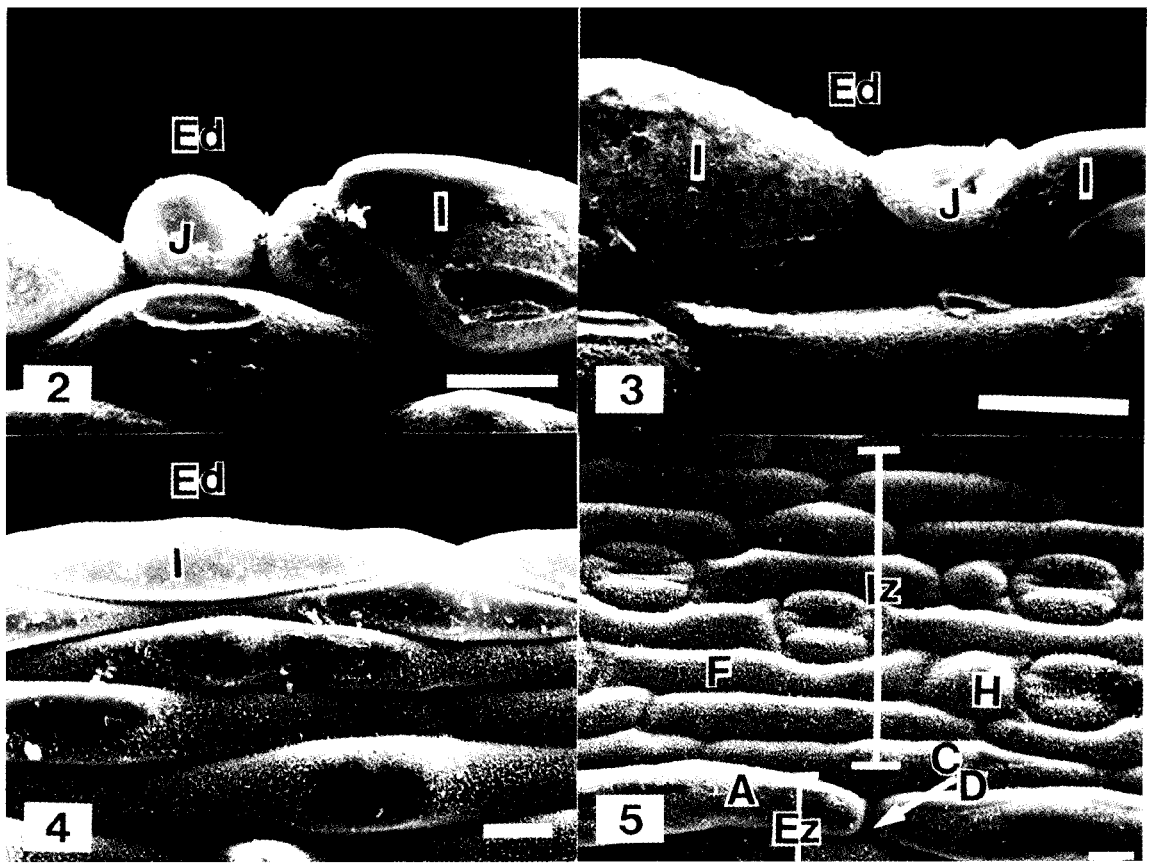
Mediciones al MO en diferentes tipos de células epidérmicas localizadas en el borde, zona internérvica y epinérvica de hojas de *D.antarctica* creciendo en la Antártida y en cámaras climáticas a 2 ± 1,5 °C y 13 ± 1,5 °C. Se entregan también de las dimensiones del estoma (células.guardianas). Los datos representan el promedio de 30 mediciones en tres hojas de diferentes plantas. Desviación estándar fue inferior al 10%. No presente (—) . Largo (L), ancho máximo (w^1), ancho mínimo (w^2). Las letras representan los diferentes tipos celulares mostrados en Fig.1

Describer	Cell Type	Plants growing at: Antarctic			Climatic chamber at: 2 ± 1.5 °C			13 ± 1.5 °C		
		L	w^1	w^2	L	w^1	w^2	L	w^1	w^2
Adaxial epidermal (μm)										
Edge	I	65.7	16.9	9.0	70.5	13.9	7.5	217.5	14.0	14.0
	J	22.4	16.9	16.9	27.1	16.1	16.1	—	—	—
Internervic	A	145.2	19.6	12.1	104.7	18.9	12.8	295.3	18.8	6.9
	E	229.0	16.2	14.2	—	—	—	—	—	—
	H	38.9	8.0	6.0	—	—	—	—	—	—
Epinervic	A	101.6	15.9	8.4	84.5	15.3	10.7	220.2	25.4	15.0
	B	113.2	17.0	12.9	—	—	—	—	—	—
	C	16.5	11.7	11.7	—	—	—	—	—	—
	D	8.9	10.3	10.3	—	—	—	—	—	—
Stomata		24.3	16.2	16.2	33.8	20.3	20.3	—	—	—
Abaxial epidermal (μm)										
Edge	I	70.9	18.0	10.0	86.1	16.0	9.0	215.9	14.8	7.0
	J	21.7	17.2	17.2	26.1	12.8	12.8	—	—	—
Internevic	A	100.5	15.9	9.3	188.1	21.9	13.2	344.9	16.8	8.1
	F	80.1	16.0	26.8	213.1	17.3	26.9	296.4	13.6	23.5
	G	—	—	—	—	—	—	38.9	8.0	8.0
Epinervic	A	122.0	28.8	16.0	202.5	36.9	18.7	294.7	28.1	14.1
Stomata		35.5	8.3	8.3	40.3	10.9	10.9	44.7	8.3	8.3

Ten plants in each treatment were used. Plants were irrigated every three days at 80% field capacity with water or nutrient solution. The leaves from the second node, collected directly in the field and those grown in the laboratory and taken from the middle of the leaf blade were used for light (LM) and scanning electron microscopy (SEM). Each sample was estimated as an experimental entity for the observations and measurements. Means of particular measurements (described below) were calculated from the individual values. The statistical differences between the means were established by an ANOVA ($P < 0.05$), and a Tukey test was performed when the distance between means was significant (Little & Hills 1976).

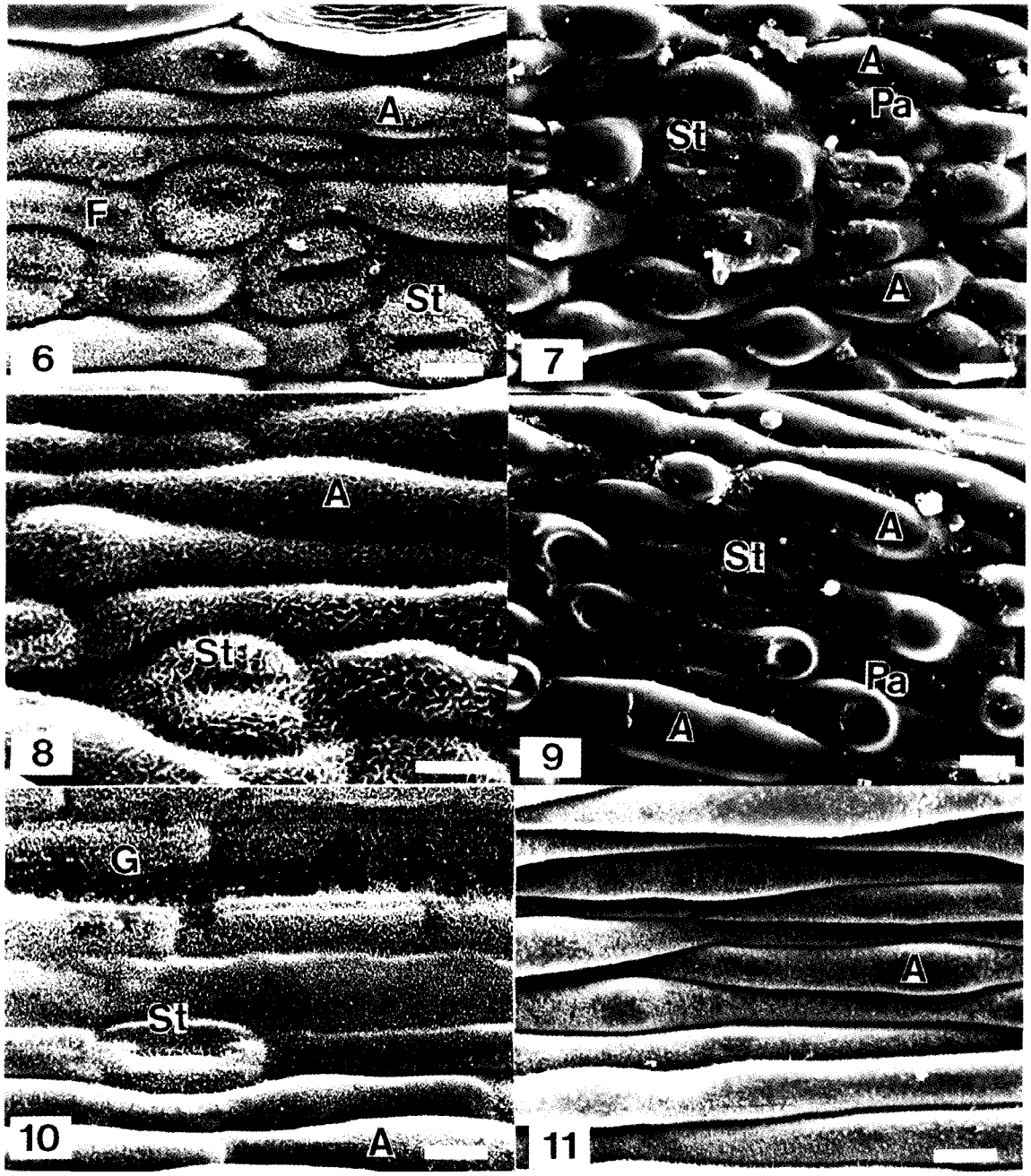
Epidermal isolation for light microscopy (LM)

Adaxial and abaxial epidermal were isolated and cleared according to Aiken & Lefkovitch (1984). Peeled leaf epidermal preparation montage was made in glycerinated-gelatin and observed by LM (Zeiss). Measurements of length and width (w^1 = widest part and w^2 = narrowest part) were



Figs. 2-5: Scanning electron microscopy (SEM) micrography of epidermal foliar peels of *D. antarctica* showing some of the cell types observed under LM, located in the edge (Ed) and in the internervic (Iz) and the epinervic (Ez) zones. Figs. 2, 3: Edge cell (Ed), type J and I of plants growing in the Antarctic. Fig. 4: Edge cell (Ed) type I of plants growing at 13°C. Fig. 5 Abaxial epidermis of plants growing in the Antarctic showing the internervic zone (Iz) with the cell types F, H and the epinervic zone (Ez) with the cell types A, C, D. Scale bar = 15 μm.

Microfotografía al microscopio electrónico de barrido (MEB) en epidermis foliar de *D. antarctica* mostrando algunos tipos celulares observados al MO, localizados en el borde (Ed), en la zona internérvica (Iz), y en zona epinérvica (Ez). Figs. 2, 3: Células del borde (Ed), tipo J e I, de plantas creciendo en la Antártida. Fig. 4: Células del borde (Ed) tipo I en plantas creciendo a 13°C. Fig. 5: Epidermis abaxial de plantas creciendo en la Antártida mostrando la zona internérvica (Iz) con células del tipo F y H y la zona epinérvica (Ez) con células del tipo A, C y D. Barra = 15μm.



Figs. 6-11: SEM of epidermal peels of leaves of *D. antarctica* growing in the Antarctic and in the laboratory at two different temperatures showing some of the cell types observed under LM. Figs. 6, 8, 10: Abaxial leaf surface. Figs. 7, 9, 11: Adaxial leaf surface. Figs. 6, 7: Samples of the Antarctic habitat with the cell types A, F, stomata (St) and papillae (Pa). Figs. 8, 9: Samples of plants growing at 2 °C showing collapsed papillae (Pa), stomata (St), and cells type A. Figs. 10, 11: Samples of plants growing at 13 °C with cell type. Stomata (St) and cell type G are present only in the abaxial surface. Note the increase of cell size with temperature. Papillae are absent. Scale bar= 15 μm.

Vista en MEB de epidermis foliar en plantas de *D. antarctica* creciendo en la Antártida y en el laboratorio a dos temperaturas diferentes, mostrando algunos tipos celulares observados al MO. Figs. 6, 8, 10: Superficie abaxial. Figs. 7, 9, 11: Superficie adaxial. Figs. 6, 7: Muestra del habitat antártico con células tipo A, F, estoma (St) y papila (Pa). Figs. 8, 9: Muestra de plantas creciendo a 2 °C con papilas colapsadas (Pa), estoma (St) y células de tipo A. Figs. 10, 11: Muestra de plantas creciendo a 13°C con células de tipo A. Estoma (St) y célula tipo G están presentes sólo en la superficie abaxial. Nótese el incremento de tamaño celular con el aumento de temperatura. Barra = 15 μm.

made from thirty cells of each different cell type, located at the edge, internervic and epinervic zones. Only some of these cell types are shown in the SEM micrographs. Additionally, cell and stomatal densities were determined.

Scanning electron microscopy (SEM)

Samples of leaves were vacuum infiltrated with Karnovsky and glutaraldehyde 25% in sodium phosphate buffer 0.2 M (pH 7.4) for two hours. The samples were rinsed with phosphate buffer 0.1 M for three-five minutes three times and dehydrated on ice in a graded acetone series, followed by critical point (Hitachi mod HCP-2) drying with liquid carbon dioxide. The samples were mounted on aluminum stubs and coated with gold in an Edwards S150 B sputter-coater. Samples were observed in a Bausch & Lomb Nanolab 2000 scanning electron microscope. Images were photographed on Polaroid 665 in Forte pan 50 film.

Leaf cross sections (LM)

Cross sections (1μm thick) of leaves fixed in Karnovsky, embedded in epon-araldite, and stained with toluidine blue (1%) were made. Free hand cross sections of leaf samples (16 μm thick) were also made. Direct LM measurements of the length and the width of cells, the thickness of the epidermis, the thickness of palisade and spongy-parenchyma layers, the total leaf thickness as well the vessel lumen area, were performed in several cross sections using an eye-piece micrometer. In addition, leaf dry weight and leaf area were determined.

RESULTS

Epidermal characteristics

In plants growing in the Antarctic, peeled epidermis from both sides of the leaf blades, specially the upper one, showed several cell sizes and forms (Table 1, Figs. 1, 2, 3, 5). A greater cell density per leaf area (around 36%; Table 2, Figs. 6, 7) was also found in these plants with respect to plants growing in the laboratory at 2 and 13 °C (Table 2, Figs. 4, 8, 9, 10, 11). Adaxial epidermal cells of plants growing in the Antarctic had a turgid appearance having some of them prominent papillae (Fig. 7). Papillae tended to disappear and collapse when growing at 2 °C (Fig. 9), being absent

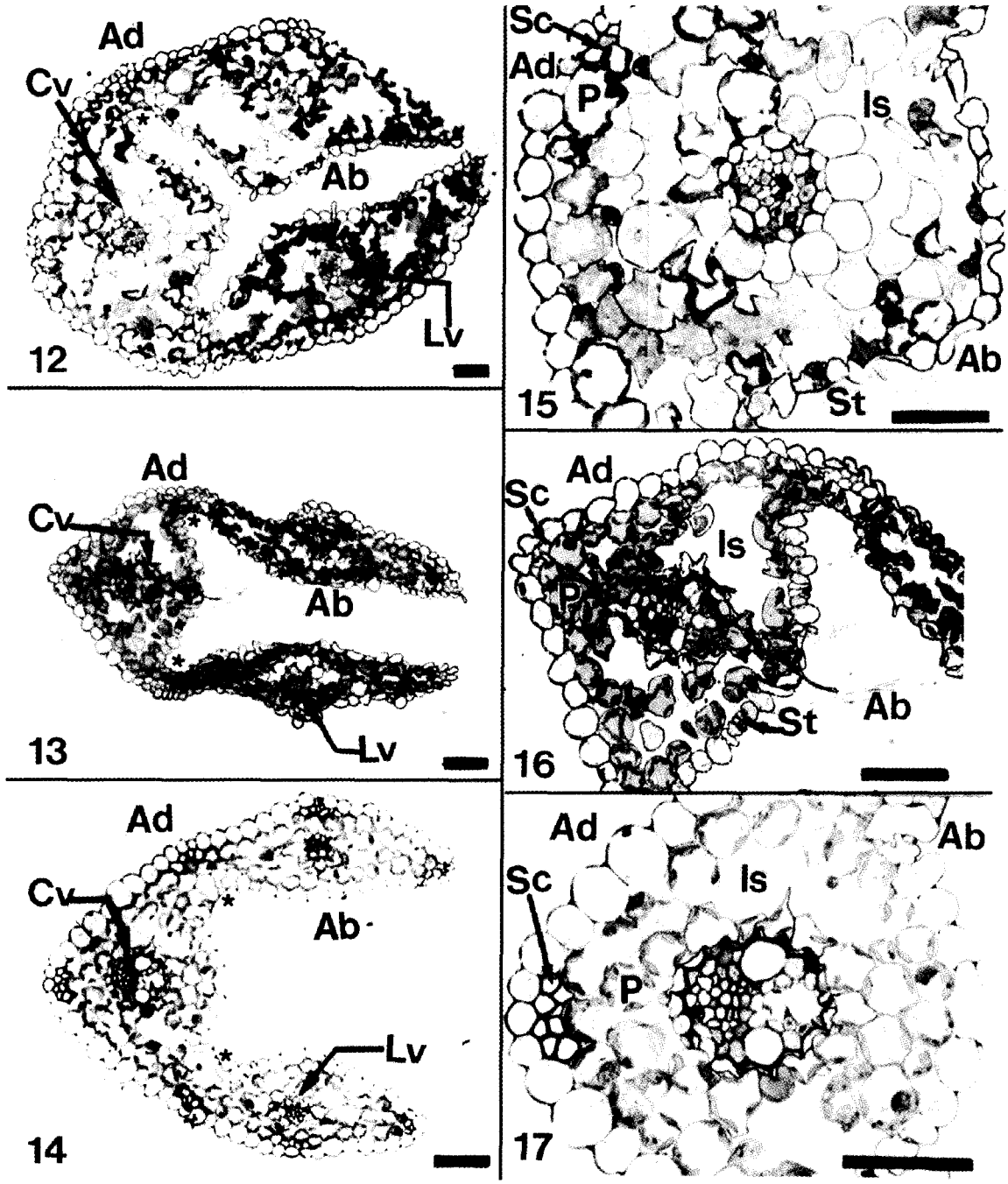
TABLE 2

LM measurements of epidermal cell and stomatal densities in peel epidermal of *D. antarctica* leaves growing at the Antarctic and in climatic chamber under two different temperatures. Stomatal index is also given. Standard deviation was lower than 10%. Not present (—)

Mediciones al MO de la densidad de las células epidérmicas y de los estomas en "peel" de epidermis de hojas de *D.antarctica* creciendo en la Antártida y en cámara climática bajo dos diferentes temperaturas. Desviación estándar fue inferior que 10%. No presente (—)

Describer	Antarctic	Plants growing at: 2 ± 1.5°C	Climatic chamber at: 13 ± 1.5 °C
Adaxial epidermal			
Epidermal cell densities (n°/m^2)	1488.40	1211.40	636.30
Stomatal density (n°/ mm^2)	170.10	78.20	—
Stomatal index*	0.11	0.06	—
Abaxial epidermal			
Epidermal cell densities (n°/mm^2)	1273.80	1021.30	371.60
Stomatal density (n°/mm^2)	382.50	278.50	92.90
Stomatal index *	0.30	0.27	0.25

*n° stomata/n° total cells x 100



Figs.12-17: LM micrographs of leaf transverse sections of *D. antarctica* showing the adaxial (Ad) and abaxial (Ab) epidermal with stomata (St), bulliform cells (*), sclerenchymatic bundle (Sc), parenchymatic cells (P), intercellular spaces (Is), central (Cv) and lateral (Lv) vascular bundle. Left: general aspect of the section. Right: middle zone. Figs. 12, 15: Plants growing in the Antarctic with the leaf blades showing strongly folded v-shaped. Figs. 13, 16: Plants growing in the laboratory at 2 °C. Figs. 14, 17: Plants growing in the laboratory at 13 °C. Scale bar = 50 μm.

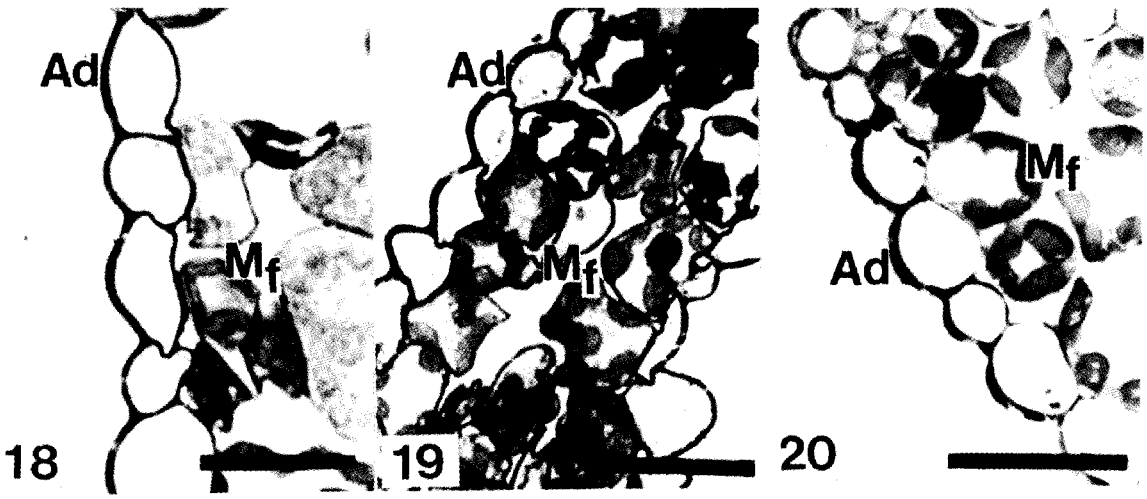
Corte transversal en MO por hoja de *D. antarctica* mostrando: superficie adaxial (Ad) y abaxial (Ab), estoma (St), células buliformes (*), banda esclerenquimática (Sc), células parenquimáticas (P), espacio intercelular (Is), haz vascular central (Cv) y lateral (Lv). Izq: Aspecto general de la sección. Der: zona media. Figs.12, 15: Planta creciendo en el hábitat antártico con la lámina foliar muy plegada en forma de V. Figs. 13, 16: Planta creciendo en el laboratorio a 2° C. Figs. 14, 17: Planta creciendo en el laboratorio a 13° C. Barra = 50 μm.

in leaves cultivated at 13 °C (Fig. 11). The complexity of cell types of the adaxial epidermal of the Antarctic samples, disappeared at 13 °C (maintaining only the cell type I and A) (Table 1). Some of these cell types are shown also in figures 7 and 11. At 13 °C the length of cells (type A) was increased by 224% in comparison to the Antarctic plants (Table 1, Figs. 7, 11). In the abaxial epidermal, the cell complexity of Antarctic samples was maintained at 13 °C with exception of the cell type J, located in the edge zone, that disappeared (Table 1, Figs. 2, 3, 4). The length of the epidermal cells of samples growing at 13 °C was increased by ca. 200% in comparison to the Antarctic samples ($P < 0.05$) (Table 1, Figs. 6, 10). Epidermal cells of plants growing at 2 °C showed intermediate sizes between the other two groups of plants (Table 1, Figs. 8, 9). Stomata were present on both epidermis, in Antarctic samples (Figs. 6, 7) and in plants growing at 2 °C (Figs. 8, 9), being more frequent in the abaxial surface. Stomata density was higher in leaves of plants growing in the Antarctic than in plants cultivated in the laboratory ($P < 0.05$) (Table 2, Figs. 6, 8, 10). In plants growing at 13 °C stomata were present only in the abaxial epidermis, being its density significantly lower than in plants growing in the Antarctic ($P < 0.05$) (Table 2, Figs. 10, 11).

Transverse sections of leaf blades

Transverse sections of leaf blades of plants growing in the Antarctic and in the laboratory at two different temperatures showed three vascular bundles (Figs. 12, 13, 14). Leaf blades of plants at the Antarctic were more folded towards the lower surface (v-shaped, Fig. 12) than the laboratory cultivated plants (Figs. 13, 14). Plants growing in the Antarctic showed a higher leaf thickness and size of the epidermal and mesophyll cells and larger intercellular spaces of the spongy parenchyma layer than leaves of the other plants ($P < 0.05$) (Tables 3, 4, Figs. 12-20). The shape of the mesophyll cells of plants in the Antarctic was irregular (Figs. 12, 15, 18), being spherical at 13 °C (Figs. 14, 17, 20).

A common feature of all investigated leaves was the presence of subepidermal strands of sclerenchymatic fibers (1 to 2 cells deep and 2 to 3 cells wide) located under the epidermis above the mesophyll cells which surround the vascular bundle in the angles of the blade edge (Figs. 15, 16,



Figs. 18-20: LM transverse section through a *D. antarctica* leaf showing the effect of the different growing conditions on the cell shapes of the adaxial epidermal (Ad) and the mesophyll (Mf). Fig. 18: Antarctic habitat. Fig. 19: Laboratory at 2 °C. Fig. 20: Laboratory at 13 °C. Scale bar= 30 μm.

Corte transversal al MO de hoja de *D. antarctica* mostrando el efecto de las diferentes condiciones de crecimiento sobre la forma de células de la epidermis adaxial (Ad) y del mesofilo (Mf). Fig. 18: Creciendo en el habitat antártico. Fig. 19: Creciendo a 2 °C. Fig. 20: Creciendo a 13 °C. Barra = 30 μm.

17). The number of cells of these strands increased concomitantly with the temperature. Bulliform cells appeared in the angles of the blade of the abaxial epidermis (Figs. 12, 13, 14). These cells are often much larger and more vacuolated than neighboring epidermal cells. They may be enabling the rolling movements of the blades (Esau 1985).

Vascular bundles.

The central and lateral vascular bundles of the Antarctic leaf samples (Figs. 15, 21, 22) showed the two differentiated bundle sheaths (an outer parenchymatous and an inner or mestome) characteristic of the Pooideae type (Esau 1985), while those of the plants growing in the laboratory at 13 °C presented only mestome (Figs. 17, 25, 26). Plants growing in the laboratory at 2 °C showed a transition state (Figs. 16, 19, 23, 24).

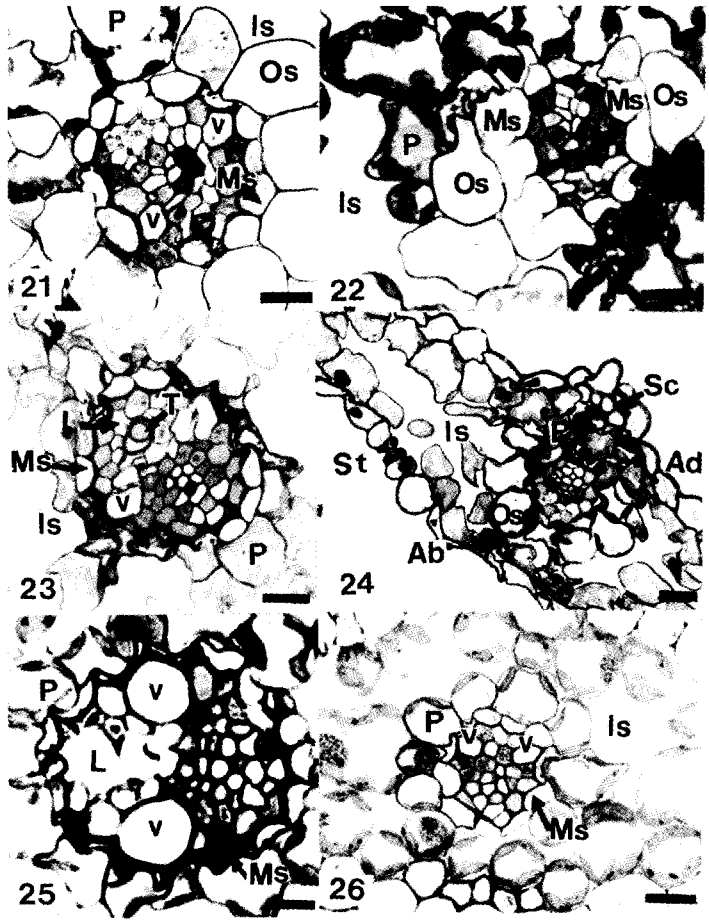
The outer bundle sheath of Antarctic leaves is incomplete and formed by thin-walled parenchymatous cells without visible chloroplasts (Figs. 12, 15, 21, 22). The cells of the outer and inner sheath of the central vascular bundle are spherical (Fig. 21) while those of the lateral bundles are very irregular in form (Fig. 22). The inner bundle sheath or mestome of leaf blades at the Antarctic is continuous and constituted by small cells with U-shape thickened walls of endodermoid type (Esau 1985). These cells reacted positively for lignin with the phloroglucin test (Sass 1958). Mestome of plants growing at 13 °C showed scarcely turgid lumen and thicker walls than those of plants growing at lower temperature (Figs. 15, 17, 21-26). Lumen vessels of vascular bundles of plants growing in the Antarctic were smaller (Fig. 22) than those of plants growing at 2 and 13 °C ($P < 0.05$) (Table 3, Figs. 23-25). The vascular bundle of the Antarctic samples did not present lacuna as the other samples (Figs. 21, 23,

TABLE 3

LM morphometric parameters in leaf transverse section of *D. antarctica* growing in the Antarctic and in climatic chamber at two differents temperatures (2 ± 1.5 °C and 13 ± 1.5 °C). Additionally, other leaf parameters are given. Not present. (—). Standard deviation (±)

Parámetros morfométricos medidos al MO en corte transversal de hojas de *D. antarctica* creciendo en la Antártida y en cámaras climáticas a dos diferentes temperaturas (2 ± 1,5 °C y 13 ± 1,5 °C). Adicionalmente se entregan otros parámetros. No presente (—). Desviación estándar (±)

Describer	Antarctic	Plants growing at: 2 ± 1.5 °C	Climatic chamber at: 13 ± 1.5 °C
Adaxial epidermal			
Thickness (μm)	20.0 ± 0.4	16.3 ± 0.6	18.3 ± 1.0
Abaxial epidermal			
Thickness (μm)	21.1 ± 0.3	17.2 ± 0.2	17.9 ± 1.0
Mesophyll			
Palisade layer thickness (μm)	28.4 ± 1.4	36.8 ± 1.2	48.4 ± 1.4
Ratio palisade/palisade + spongy	0.1	0.3	0.4
Spongy layer thickness (μm)	180.6 ± 2.6	100.0 ± 2.4	60.5 ± 3.0
Number of vascular bundles	3.0	3.0	3.0
Vessel elements			
Lumen area (μm^2)	80.0 ± 4.2	99.4 ± 2.4	157.0 ± 6.1
Lumen area increase (%)	—	24.3	96.3
Other characteristics			
Leaf thickness (μm)	250.3 ± 3.5	188.4 ± 2.5	164.2 ± 2.0
Leaf dry weight (% PF)	25.6	22.0	19.4
Total leaf area (mm^2)	30.1 ± 4.8	145.3 ± 11.6	184.1 ± 16.4
Leaf area increase (%)	—	382.7	511.6



Figs. 21-26: LM leaf transverse section of *D. antarctica* showing the central (left side) and lateral (right side) vascular bundle. Fig. 21: Samples of plants growing in the Antarctic showing the central vascular bundle surrounded by two sheaths: an outer of parenchymatous cells without chloroplasts (Os) and an inner sheath or mestome with thickened internal cell walls (Ms), small lumen vessels of the metaxylem (V). Fig. 22: Antartic samples. Lateral vascular bundle with very irregular cell forms in the outer sheat (Os) and inner sheath or mestome (Ms). Fig. 23: Central vascular bundle of plants growing at 2 °C without outer sheaths showing the mestome (Ms), vessels (V), tracheary elements of protoxylem (partially destroyed) with an anular tracheid (T) and a small lacuna (L). Fig. 24: Samples at 2 °C. Lateral vascular bundle showing a transition state of the outer sheath (Os) with chloroplasts, and a reduced mestome (Ms). Adittionally the adaxial (Ad) and abaxial (Ab) epidermal, intercellular space (Is), stomata (St) and sclerenchymatic bundle (Sc) are shown. Fig. 25: Samples at 13 °C showing the central vascular bundle without outer sheaht and a mestome (Ms) with some collapsed cells, two very differentiated tracheary elements (V) and a great lacuna (L). Fig. 26: Samples at 13 °C showing the mestome (Ms) of lateral vascular bundle with cell walls thinner than those of Antarctic samples. Scale bar=15 μm. Note the increase of the lumen vessels and lacuna with the temperature increase.

Corte tranversal en MO por hoja de *D. antarctica*, mostrando el haz vascular central (lado izquierdo) y lateral (lado derecho). Fig. 21: Muestra foliar de la Antártida con el haz vascular central (Cv) rodeado por dos vainas: una externa de células parenquimatosas sin cloroplastos (Os) y una interna o mestoma (Ms) con la pared celular interna engrosada, vasos (V) del metaxilema de lumen pequeño. Fig. 22: Muestra Antártida. Haz vascular lateral (Lv) con células de forma irregular en ambas vainas: la externa (Os) y la interna o mestoma (Ms). Fig. 23: Haz vascular central (Cv) de plantas creciendo a 2 °C, sin vaina externa, mostrando mestoma (Ms), vasos (V), elementos traqueidales del protoxilema (parcialmente destruídos) con traqueida (T) anular y una pequeña laguna (L). Fig. 24: Muestra foliar a 2 °C. Haz vascular lateral (Lv) mostrando la vaina externa (Os) con cloroplastos (estado de transición) , y un reducido mestoma (Ms). Además, se observa: la superficie adaxial (Ad) y abaxial (Ab), espacio intercelular (Is), estoma (St) y banda esclerenquimática (Sc). Fig. 25: Muestra a 13 °C mostrando el haz vascular central (Cv) sin vaina externa y un mestoma (Ms) con algunas células colapsadas, dos elementos traqueales muy diferenciados (V) y una gran laguna (L). Fig. 26: Muestra a 13°C mostrando el mestoma (Ms) del haz vascular lateral (Lv) con la pared celular más delgadas que las de la muestras antártica. Barra=15 μm. Nótese el incremento de tamaño del lumen de la laguna y vasos con el incremento de temperatura.

25). The annular tracheids, only appears at 2 °C (Fig. 23). The dimensions of the lacuna in samples growing in the laboratory increased with the growth temperature (Figs. 23, 25).

DISCUSSION

Our results showed that leaf anatomical characteristics of specimens of *D. antarctica* growing in the Antarctic, in comparison with those growing in the laboratory (at 2 and 13 °C), were strongly xerophytic (e.g. smaller epidermal cell sizes, higher cell density and complexity of cell forms, thicker cuticle, higher stomata density, greater leaf thickness, smaller lumen vessels). In these habitats, as well in other cold regions, low temperature can induce drought stress and thus xerophytic leaf structures (Körner & Larcher 1988). The changes of the majority of these characteristics when plants grew in the laboratory reflected modifications not consistent with the reduced intraspecific morphologic variability of the epidermis of the Poaceae (Esau 1985). These results suggest that the taxonomic value of epidermal characteristics as proposed by Upadhyaya & Furnes (1994) may not be applicable to all plant groups, specially when members of the Poaceae are considered. The presence of papillae in *D. antarctica* growing in their natural habitat could be interpreted as a storage mechanism of carbohydrates involved in freezing point depression, as reported for other plants (Levitt 1980, Sakai & Larcher 1987, Larcher 1995). That is consistent with the high contents of sucrose and fructans found in *D. antarctica* growing in the Antarctic (Zúñiga et al.

TABLE 4

LM cytological leaf parameters (high, width, area and ratio width/high) of *D. antarctica* growing in the Antartic and in climatic chambers at 2 ± 1.5 and 13 °C ± 1.5. Measurements were made under LM in leaf transverse sections. Data represent means of 10 leaves of different plants. Standard deviation (±)

Parámetros citológicos (alto, ancho, área y radio ancho/alto) en hojas de *D.antarctica* creciendo en la Antártida y en cámara climática a 2 ± 1,5 °C y 13 ± 1,5 °C. Mediciones realizadas al MO en cortes transversales de hoja. Los datos representan al promedio de 10 hojas de plantas diferentes. Desviación estándar (±)

Describer	Plants growing at: Antarctic	2 ± 1.5 °C	Climatic chamber at: 13 ± 1.5 °C
Adaxial epidermal cells (μm)			
High	16.8 ± 0.5	12.4 ± 0.4	18.4 ± 0.3
Width	14.8 ± 0.6	11.6 ± 0.3	15.6 ± 0.5
Area (μm²)	248.6	143.8	287.0
Ratio width/high	0.9 ± 0.5	0.9 ± 0.4	0.8 ± 0.4
Abaxial epidermal cells (μm)			
High	20.8 ± 0.9	13.6 ± 0.5	18.8 ± 0.6
Width	11.2 ± 0.4	9.6 ± 0.3	15.2 ± 0.4
Area (μm²)	233.0	130.6	285.8
Ratio width/high	0.5 ± 0.5	0.7 ± 0.4	0.8 ± 0.5
Palisade parenchyma cells (μm)			
High	24.0 ± 1.1	15.0 ± 0.3	16.8 ± 0.2
Width	20.0 ± 1.9	14.8 ± 0.2	16.0 ± 0.3
Area (μm²)	480.0	222.0	268.8
Ratio width/high	0.8 ± 1.5	1.0 ± 0.1	1.0 ± 0.1
Spongy parenchyma cells (μm)			
High	32.6 ± 2.1	21.2 ± 2.0	25.1 ± 1.3
Width	22.9 ± 1.0	19.6 ± 1.0	21.1 ± 0.8
Area (μm²)	883.9	415.5	529.6
Ratio width/high	0.7 ± 1.6	0.9 ± 1.5	0.8 ± 1.1

1996), although their localization in the papillae remains to be established. Fructans are often related with cold hardiness of plants (Pontis & Del Campillo 1985, Livingston III & Henson 1998). The high frost resistance (LT_{50} around –27 °C) of *D. antarctica* (Casanova 1997) could be related, at least partially, with these substances. Fructans participate also in the osmoregulation of cells in periods of limited water availability (Virgona & Barlow 1991, Pollock & Cairns 1991, Hendry 1993).

The v-shaped position of leaf blades of plants at the Antarctic habitat are similar to the typical graminoid leaves of both, high mountains and polar tundra ecosystems (Körner & Larcher 1988). They could serve as an isolation leaf strategy, conferring independence with respect to the environment, maintaining the stomata of the abaxial surface under a high relative humidity, thus decreasing the transpiration rates and maintaining the gas exchange (Larcher 1995, Hardy et al. 1995). Zúñiga et al. (1996) found increased leaf temperatures with respect to the air temperatures in *D. antarctica* growing in the Antarctic which could be produced by low transpiration rates in this species. It is known, that in non transpiring plants the temperature of leaves increased in various grades with respect to air temperatures (Larcher 1995). Under these conditions the photosynthesis could be maintained by CO_2 accumulation in the abundant intercellular spaces of the mesophyll. The irregular mesophyll cell forms of plants growing in the Antarctic are not an artifact of the fixation method because we observed the same mesophyll cell forms in fresh preparations (not shown). It is reported that the irregular mesophyll cell forms may provide an important interface favoring a more intensive CO_2 gas exchange (Nobel & Walker 1985, Körner & Larcher 1988, Upadhyaya & Furness 1994). The maximum stomatal conductance for CO_2 uptake and loss of water vapor is greater in leaves with stomata present on both surfaces than on the lower surface only (Beerling & Kelly 1996). This morphologic characteristic could improve the water absorption under water stress induced by low temperature (Larcher 1995). Our results showed a relationship between the leaf thickness, the occurrence of amphistomaty (stomata present on the upper and lower surfaces) and Antarctic habitat with high irradiance. These results support the suggestions by Mott et al. (1982) about the occurrence of amphistomatic, bifacial and thicker leaves in plants exposed to full sun. Indeed, thick and amphistomatic leaves can facilitate CO_2 diffusion to the mesophyll cells on each side of a leaf (Nobel & Walker 1985).

Sclerenchymatic bands may provide an advantageous characteristic to the leaves of plants growing in unfavorable habitats with regard to a possible disturbance of water conduction, conferring strength to the mesophyll and preventing collapse of leaf tissues under water stress (Pyykkö 1966). With respect to the high xerophytic characteristics of the Antarctic samples, we had expected a high development of their sclerenchymatic tissues. Unexpectedly, they were scarcely developed with respect to plants growing in the laboratory at 13 °C.

The presence of two bundle sheaths (an outer and an inner) in the vascular bundles of leaves of some plants has been associated with an adaptation to a high radiation intensity (Brown 1958, Pyykkö 1966). This structural feature was also present in leaves of *D. antarctica* growing in the Antarctic where also high photon flux densities are expected (Casanova, 1997). Recent ultrastructural studies in this plant material revealed the presence of inconspicuous chloroplasts in this sheath (own unpublished results). This feature resembles the "Kranz syndrome" of some Gramineae, where reduced grana are present (Smith & Brown 1973). These results could suggest a functional specialization to optimize photosynthetic success under the harsh Antarctic environmental conditions as reported for other plants by Hall & Langdale (1996). These authors suggest, that the optimization of the photosynthesis to different environmental stress conditions, requires the development of one or more photosynthetic pathways as occurs in the genus Flaveria where a progression of photosynthetic types from C_3 to C_4 occurs (Dai et al. 1996).

Measurements with carbon isotope (ratio of stable isotopes ^{13}C to ^{12}C in dry matter) according Smith & Brown (1973) have revealed, that *D. antartica*, as expected, does not correspond to a C_4 plant. The $\delta^{13}C$ value was -28.3‰ (own unpublished results) typical for a C_3 plant (refs. in Larcher 1995). Thus, according to the structural features of vascular bundles of *D. antarctica* could present, depending on the environmental conditions, some of the different C_4 photosynthetic subtypes mentioned by Hall & Langdale (1996) and Dai et al. (1996). The absence of the outer bundle sheath in plants growing in the laboratory at 13 °C and 180 µmol m^{-2} s^{-1} supports the assumption that this structure and function could be more associated to the high irradiance of the Antarctic habitat, than to low temperature. More studies with respect to the photosynthetic enzyme types and their compartmentalization in this plant growing under different environmental conditions are need.

The survival capacity to freezing conditions of *D. antarctica* growing in the Antarctic could be ensured by a more developed mestome. Mestome may provide, as in other plants, resistance to plasmolysis, because of the intimate contact of cell content with the thick walls (Esau 1985). It favoured, also the depression of the freezing water point (Kaku 1971, Huner 1985). The small area of the lumen of vessels can also confer resistance to low temperature in the Antarctic because water freezes slowly in smaller than in greater area vessels (Ashworth & Abeles 1984).

Most of the leaf characteristics found in plants growing in the Antarctic have been described for plants from other cold areas (Körner & Larcher 1988, Consaul & Aiken 1993). Our results suggest a plastic modification (sense Pyykkö 1966) of leaves of *D. antarctica* to the harsh Antarctic habitat. In addition, the strong tussock graminoid growth form of *D. antarctica* in the Antarctic (Casaretto et al. 1994) disappeared and the leaf surface increased in plants growing in the laboratory, although their inner surface decreased. All these changes showed that *D. antarctica* can loose some of the anatomical characteristics acquired in the harsh Antarctic habitat when brought to milder conditions. Therefore, our assumption about a genotypic adaptation of anatomical leaf features of *D. antarctica* to colder temperatures is not proved, specially with the disappearance of the outer sheath of vascular bundle and the increase in the vessel lumens at increasing temperatures (13 °C). Additional factors, not reproduced in the climatic chamber, but present in the natural habitat, may be responsible for the anatomical variations between plants growing in the Antarctic and in the laboratory. For example, the quality and quantity of the light in the growth chamber and in the natural habitat were different. While total incoming radiation under the full midday sun can reach around 2000 µmol m^{-2} s^{-1} in the Antarctic (Edwards & Lewis-Smith 1988), in our growth chamber the light intensities were ca. 180 µmol m^{-2} s^{-1}. The light quality was also different in the Antarctic compared to the light in the climatic chamber (fluorescent light source).The possible effects of increased UV-B light in the Antarctic on *D. antarctica* are yet to be studied. Becwar et al. (1982) reported that the leaf structure of plants growing at high altitude was modified when compared to leaves growing in chambers, with a loss of radiation of about 38 to 50%, with respect to natural habitats. High radiation also brings secondary effects (heat and water deficit) that may change the response of the plant to local light intensity (Larcher 1995). These effects, specially water deficit, could also be expected as consequence of low temperatures in the Antarctic habitat during the growing season. The experiments in the laboratory were made under uniform environmental conditions (light intensities, relative humidity, irrigation) varying only the growth temperature. They showed that the xerophytic leaf features of plants of *D. antarctica* decreased when growing at a higher temperature.

With respect to regional warming in the Antarctic, Lewis-Smith (1994) pointed out that the increase in populations of *D. antarctica* and *Colobanthus quitensis* (Kunth) Bartl. in this region, could be used as a valuable bioindicator. Our study provides a complement for future evaluations of Antarctic climatic warming. It provides

information about the present leaf anatomical characteristics of *D. antarctica* and the variation possibilities under more moderate conditions. Finally, these results indicate that the leaf anatomical characteristics of *D. antarctica* are not an adaptation produced by natural selection under harsh Antarctic conditions, but rather a plastic response of the phenotype to these conditions.

ACKNOWLEDGMENTS

We thank Prof. Dr. Peter Weinberger for reviewing the manuscript and to Instituto de Histología, Universidad Austral de Chile, Valdivia, Chile, for the use of the scanning electron microscope and for the training and the advising on the SEM. This work was supported by DID-UACH S- 95-39, FONDECYT 1970637 and 1980967, and Instituto Antártico Chileno (INACH-148).

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