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Sugars and enzyme activity in the grass *Deschampsia antarctica*

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Abstract: *Deschampsia antarctica* is a freezing-tolerant plant and the only native Poaceae that grows in the Maritime Antarctic. During the long days of the growing season this plant accumulates sucrose (Suc) in the leaves to 36% of the dry weight. The mechanism that leads to this high accumulation is unknown. The effect of day length and low temperature on sucrose phosphate synthase (SPS) (EC: 2.4.1.14) activity and sugar accumulation was studied in *D. antarctica* and compared with other Poaceae. Three different day lengths: short (SD) (8/16 h), medium (MD) (16/8 h) and long (LD) (21/3 h); and two temperatures: 4°C (cold-acclimated) and 15°C (non-acclimated) were tested. The highest contents in total soluble sugars (TSS) and Suc were reached in crowns and leaves, respectively, in cold-acclimated plants under LD. TSS and Suc contents and SPS activity with cold acclimation were higher in *D. antarctica* than in other agricultural (wheat, oat and barley) and non-agricultural (*D. caespitosa* and *D. beringensis*) Poaceae species. Suc/TSS ratio was higher in all *Deschampsia* species than in agricultural Poaceae species. SPS activity and sucrose content in leaves were positively correlated only in LD cold acclimated plants. This result shows that SPS activity is responsive to day length in *D. antarctica*.

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Key words: cold acclimation, maritime Antarctic, Poaceae, sucrose accumulation

Introduction

Cold-resistant plants have different survival strategies under harsh conditions. One of these is sugar accumulation, which has been reported for many plants during cold acclimation (Koster & Lynch 1992). Plants that grow in temperate zones may also accumulate fructans (Vijn & Smeekens 1999). Fructans (poly-fructosylsucrose) are the main storage carbohydrate in grasses, including major crops, such as wheat and barley (Morvan-Bertrand *et al.* 1999, Muller *et al.* 1999). High fructan and sucrose (Suc) contents are correlated with cold tolerance in Poaceae species (Hurry *et al.* 1995). It has been suggested that fructans may act as cryoprotectants that influence cold hardiness of fructan-accumulating species (Eagles 1967, Pontis 1989). Low temperature can stimulate Suc accumulation in many plants (Alberdi & Corcuera 1991). Consequently, high Suc content is frequently observed in cold-acclimated plants (Sicher & Kremer 1984). Suc is related to cryoprotection *in vitro* (Lineberger & Steponkus 1980, Newsted *et al.* 1991) and macromolecular stabilization (Arakawa & Timasheff 1982, Strauss & Hauser 1986). Studies of photosynthetic acclimation of over-wintering cereals have established a strong correlation between freezing tolerance and the ability to maintain photosynthesis and accumulate sugars (Hurry & Huner 1991, Huner *et al.* 1993, Oquist *et al.* 1993). Increments in Suc concentration in leaves may be the result of low transport, low consumption or even high synthesis. For example, low temperature for a short period leads to low transport rates and to an increment in Suc content in leaves of *Arabidopsis thaliana* (Strand *et al.* 1999).

There are two enzymes systems directly involved in sucrose biosynthesis. The first one is sucrose phosphate synthase (SPS, EC: 2.4.1.14) that catalyzes the reaction UDP-Glc + Fru-6-P = sucrose phosphate + uridine diphosphate. SPS is thought to play a major role in sucrose biosynthesis (Huber & Huber 1996). The hydrolysis of sucrose phosphate is catalyzed by sucrose phosphate phosphatase (SPP, EC: 3.1.3.24) which renders the synthetic reaction irreversible in favour of sucrose accumulation (Wang *et al.* 2000). Some evidence suggests that SPS and SPP associate to form a multienzyme complex (Echeverria *et al.* 1997). The second enzyme is sucrose synthase (SS, EC: 2.4.1.1.13), which catalyses a reversible reaction UDP-Glc + Fru = sucrose + UDP. SS is thought to catalyze sucrose degradation for synthesizing UDP-Glc (Heim *et al.* 1993). SPS, a key enzyme in sucrose biosynthesis (Huber & Huber 1996, Jones & Ort 1997) in some species, such as tomato, corn, barley, and spinach, is regulated indirectly by light, presenting high activity during the light period and low activity in the dark (Sicher & Kremer 1984, Stitt *et al.* 1988, Huber & Huber 1990, Jones & Ort 1997).

There are only two vascular plants that have colonized the Maritime Antarctic. One of them, *Deschampsia antarctica* Desv. is a freezing-tolerant grass (Bravo *et al.* 2001) which grows during the summer, when the average air temperature is near 2ºC (Zúñiga *et al.* 1996), with an estimated day

length at the collection site (62ºS) that ranges from about 19 h at the end of December to 13 h at the beginning of March (Kirk 1994). *Deschampsia antarctica* is usually covered by snow from April until November. It is not known whether the leaves survive the winter. In the field, *D. antarctica* has an unusually high content of Suc, reaching up to 36% of dry weight (Zúñiga *et al.* 1996). This high amount of Suc found in *D. antarctica* may be the result of high Suc biosynthesis. We hypothesized that long day conditions in the Antarctic summer result in higher SPS activity, leading to higher Suc accumulation. The objectives of this study were:

a) to determine the effect of day length and low temperature on SPS activity and sugar accumulation in *D. antarctica*, and

b) to compare the effect of the same treatments on wild *D. caespitosa* and *D. beringensis*, that grow at a similar latitude in the Northern Hemisphere to *D. antarctica*, and on cultivated (wheat, oat and barley) Poaceae.

Materials and methods

Plant material and growth conditions

Deschampsia antarctica Desv. (Poaceae) was collected from the Coppermine Peninsula on Robert Island, South Shetland Islands, Maritime Antarctic (62º22'S; 59º43'W). A description of the environmental conditions of the habitat has been published elsewhere (Alberdi *et al.* 2002, Zúñiga *et al.* 1996). Plants were reproduced vegetatively from ramets in plastic pots, using a rich organic soil: peat mixture (3:1) and maintained at 13–15ºC in a growth chamber (Forma Scientific Inc., Marietta, OH, USA) with a photon flux density of 100–120 µmol m^{-2} sec^{-1} at the top of the canopy and a 16/8 h light/dark period. Plants were fertilized with Phostrogen® (Solaris, Buckinghamshire, UK) using 0.2 g l^{-1} once every two weeks.

Other Poaceae species were used to compare Suc content in leaves: wheat (*Triticum aestivum* L. cv Renaico, a spring cold resistant cultivar), oat (*Avena sativa* L. cultivar Urano), barley (*Hordeum vulgare* L. cultivar Libra), *Deschampsia caespitosa* (L.) P. Beauv. and *Deschampsia beringensis* Hulten. Seeds of *D. caespitosa* and *D. beringensis* were produced in the field in south Iceland (64ºN) from plants collected in Iceland and Alaska, respectively. Plants were grown from seeds under the same conditions as described above. Cold acclimation treatments consisted in transferring plants to other growth chambers set at 4ºC with the same light intensity and three different day lengths 8/16 h = SD, 16/8 h = MD and 21/3 h = LD. While the cold treatments were carried out, control plants were kept at 15ºC and different day lengths: (SD, MD and LD). These treatments correspond to non-acclimated conditions. Plants acclimated at SD and LD were transferred for one week to the new day length conditions before the experiment started.

Sugar extraction

Samples of mature leaves (fully expanded leaf blades), young leaves (not fully expanded leaf blades), crowns (1 cm basal zone, including leaf sheaths and meristem) and roots (mostly white live roots) of *D. antarctica* and samples of mature leaves of other species were homogenized and extracted with ethanol (86%) for 24 h, and then centrifuged at 12 000 g for 10 min. Three independent samples of 0.01 g of fresh tissue were randomly collected from around 10–20 ramets. The supernatant was depigmented in a mixture of 1:3 (v/v) with chloroform, and the aqueous fraction was freeze-dried overnight. The dry residue was resuspended in 500 µl of methanol. Total soluble sugar (TSS) content was determined spectrophotometrically by the Resorcinol method (Roe 1934) at 520 nm, using Suc as a standard. Xylose was used as a standard to calculate carbohydrate losses during the extraction procedure. Xylose recovery was 90 ± 2%.

Suc content was determined by high performance thin layer chromatography (HPTLC) using a Silicagel plate 60 F 254 Merck, pretreated with a 0.1 M methanolic potassium phosphate solution. HPTLC plates were developed five times in acetonitrile:water (85:15 v/v). Standards of 0.1 mg ml^{-1} of Suc, glucose and fructose (Merck) were used for the calibration curves (Lee *et al.* 1979). The position of Suc was visualized by the diphenylamine/aniline reagent at 105ºC and the plate was scanned at 520 nm.

Dry weight at different day lengths

Groups of 20 ramets of the same number of leaves were separately cultivated for 21 days at different day lengths (SD, MD and LD) and 15º or 4ºC. The radiation integrals received by the plants under these three conditions were SD = 2.88 mol m^2 day^{-1}, MD = 5.76 mol m^2 day^{-1} and LD = 7.56 mol m^2 day^{-1}. After 21 days, the samples were harvested; each ramet was separated into shoots and roots, weighed and then dried at 60ºC. Samples were weighed after one day of drying and then kept for around two weeks until constant weight was obtained.

Enzyme extraction

Samples of fresh tissue were frozen in liquid nitrogen and stored at -80ºC until used. In each determination three independent samples of mature leaves were used. The samples were ground to a fine powder using mortar and pestle with liquid nitrogen and suspended in extraction buffer (0.15 g of tissue per 900 µl buffer), consisting of 40 mM Hepes (pH 7.5), 15 mM $MgCl_2$, 10 mM dithiothreitol (DTT), 2% w/v polyethylene glycol, 0.1% Triton X-100 and benzamidine 2 mM. The homogenized tissue was subsequently centrifuged at 12 000 g at 4ºC and the supernatant was desalted and depleted of endogenous

substrates on a G-25 Sephadex column that had been previously equilibrated in assay buffer consisting of 40 mM Hepes (pH 7.5), 15 mM $MgCl_2$ and 1 mM DTT.

Enzyme assay

SPS activity was measured under non-limiting conditions in a reaction mixture, which contained 40 mM Hepes (pH 7.5), 15 mM $MgCl_2$, 1 mM DTT, 40 mM glucose 6-phosphate, 10 mM UDP-glucose, 20 mM fructose 6-phosphate. Uridine 5'-diphosphate, one of the products formed, was determined by a NADH consuming multi-enzyme reaction (pyruvate kinase [7.2 U ml^{-1}], lactate dehydrogenase [11.3 U ml^{-1}]), using 0.8 mM phosphoenol pyruvate and 0.3 mM NADH as substrate. SPS activity was measured at 30ºC following the decrease in absorbance at 340 nm by consumption of NADH (Hauch & Magel 1998). The values are expressed in U g^{-1} dry weight (1 U represents the oxidation of 1 µmol of NADH per minute at 30ºC and pH 7.5)

Statistics

The statistical differences in TSS and Suc contents of cold-acclimated and non-acclimated plants in three day lengths (SD, MD and LD) and four different parts of the plants (mature leaves, young leaves, crown, and roots) were calculated using three way ANOVA. The level of significance was $P \leq 0.05$. Statistical differences in Poaceae SPS activity of cold-acclimated and non-acclimated plants in two day lengths (MD, LD) were normalized using ln function prior to the two way ANOVA (level of significance was $P \leq 0.05$). A Tukey test was then used to identify those means with significant differences. The correlations between dry mass of *D. antarctica* plants, radiation integral, SPS activity and sucrose content were determined by the Pearson test ($P \leq 0.05$).

Results

Sugar content

Day length, temperature, and time of cold acclimation significantly affected TSS and Suc content in different tissues in *D. antarctica* as determined by the three way ANOVA. We compared TSS content in different parts of the plant (mature leaves, young leaf, crown and roots) after 21 days of growth at 4ºC or 15ºC at different day lengths (SD, MD and LD). Crowns had a higher TSS content in non-acclimated and cold-acclimated plants when compared with other tissues analysed (Fig. 1). TSS content in crowns was about two to five times higher than in roots. Under SD

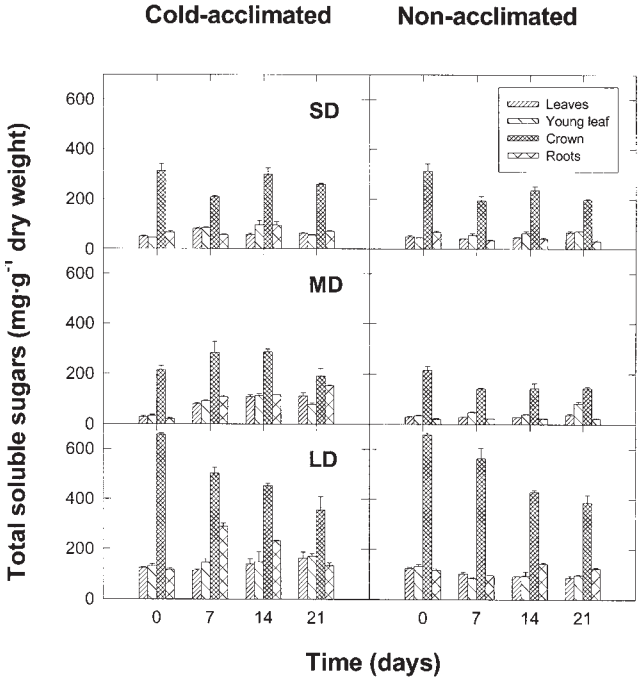

Fig. 1. Total soluble sugar content of *Deschampsia antarctica.* Plants were maintained under three day length treatments: SD = short day (8/16 h light/dark); MD = medium day (16/8 h light/dark) and LD = long day (21/3 h light/dark). Non-acclimated plants were kept at 15ºC; plants were cold-acclimated at 4º for 21 days. Values are means ± SE (vertical bars) of three separate experiments.

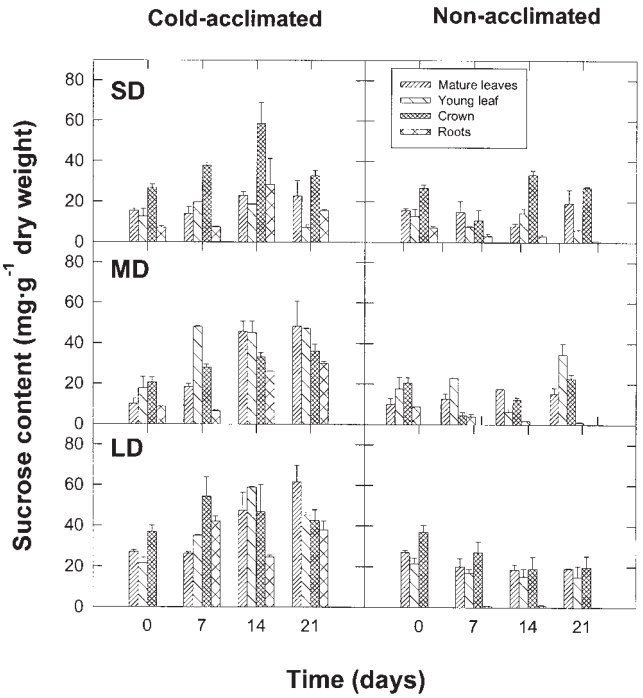

Fig. 2. Sucrose content of *Deschampsia antarctica.* Plants were maintained under three day length treatments: SD = short day (8/16 h light/dark); MD = medium day (16/8 h light/dark) and LD = long day (21/3 h light/dark). Non-acclimated plants were kept at 15ºC; plants were cold-acclimated at 4º. Values are means ± SE (vertical bars) of three separate experiments.

Table I. Suc/TSS ratio in leaves of Poaceae species. Plants were maintained at 15ºC (non-acclimated) or at 4ºC (cold-acclimated) for 21 days under three different day lengths SD = short day (8/16 h light/dark), MD = medium day (16/8 h light/dark) and LD = long day (21/3 h light/dark). Values are mean ± SE of three separated experiments. NM = not measured. ND = not detected. Statistical differences were determined by the Tukey Test ($P = 0.05$). Different letters represent significant differences between species in each day length. Suc = sucrose, TSS = Total soluble sugars.

	Suc/TSS					
	SD		MD		LD	
Poaceae species	4º	15º	4º	15º	4º	15º
D. antarctica	0.15 ± 0.01a	0.32 ± 0.01c	0.27 ± 0.01b	0.55 ± 0.02e	0.42 ± 0.02b	0.22 ± 0.009g
D. beringensis	NM	NM	0.39 ± 0.02a	0.37 ± 0.01a	0.73 ± 0.04a	0.48 ± 0.02h
D. caespitosa	NM	NM	0.44 ± 0.04a	0.54 ± 0.09e	0.15 ± 0.01c	0.21± 0.007g
Barley cv. Libra	0.16 ± 0.01a	0.03 ± 0.001d	0.16 ± 0.01c	0.10 ± 0.003f	0.05 ± 0.005d	0.09 ± 0.005i
Oat cv. Urano	0.12 ± 0.007b	0.02 ± 0.006d	0.24 ± 0.01b	0.06 ± 0.001g	0.18 ± 0.01e	0.02 ± 0.005j
Wheat cv. Renaico	0.09 ± 0.006b	ND	0.04 ± 0.003d	0.03 ± 0.001h	0.11 ± 0.008f	0.26 ± 0.02g

conditions, TSS content in leaves was similar to roots and crown content was around five times higher than in leaves. For non-acclimated plants under MD we observed that the crown content was around six times higher than roots and leaves and at 21 days in cold-acclimated plants crown and roots content of TSS are non significant different. The TSS content of crowns showed no significant differences between SD and MD cold-acclimated plants. The highest amount of TSS was found in crowns at zero time of cold acclimation in LD plants (657 ± 6.3 mg g^{-1} dry weight). The change in day length treatment from MD to LD for one week increased crown TSS content around three times. The combined treatment of cold and day length decreased crown TSS content and increase distribution of TSS in the other tissues (leaves and roots). In general, the Suc content was lower in non-acclimated than in cold-acclimated plants (Fig. 2). Mature leaves contained around 1.5 times more Suc than roots at the end of the acclimation period under MD and LD conditions. We observed significant differences in the Suc content of leaves at 21 days of cold acclimation between SD and LD and between MD and SD conditions, but not between MD and LD cold-acclimated plants. The highest Suc contents were found in the leaves of cold-acclimated plants under LD (61.5 ± 8.3 mg g^{-1} dry weight) and MD (48 ± 12.4 mg g^{-1} dry weight) conditions (Fig. 2).

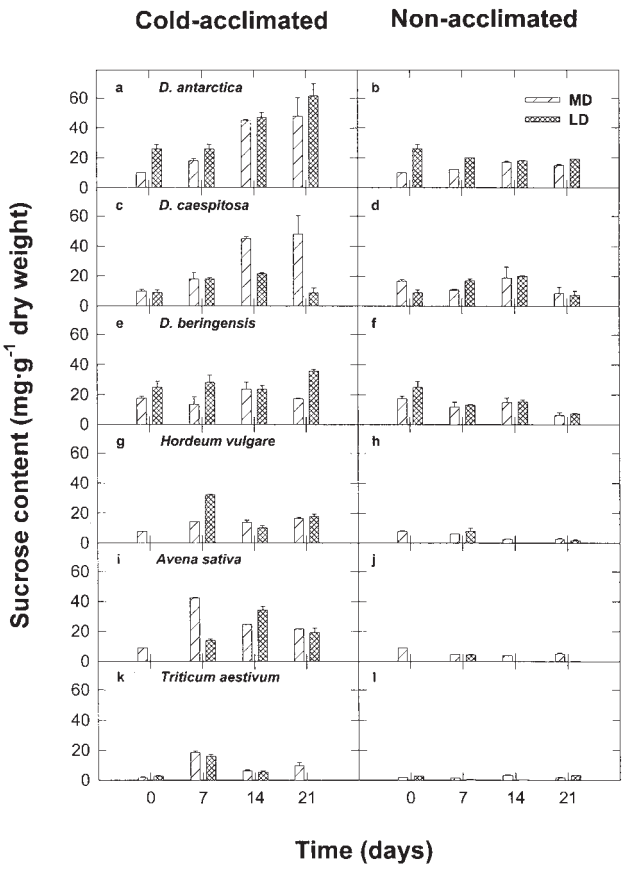

Fig 3. Leaf sucrose content in cold-acclimated (**a, c, e, g, i, k**) and non-acclimated Poaceae species (**b, d, f, h, j, l**) under two day length treatments. MD = medium day (16/8 h light/dark) and LD = long day (21/3 h light/dark). Non-acclimated plants were kept at 15ºC; plants were cold-acclimated at 4º. Spaces without draft bars represent undetected contents of sucrose. Values are means ± SE (vertical bars) of three independent experiments.

Because leaves were the part of the plant that usually accumulated the most Suc upon cold acclimation, we compared the Suc content in leaves of several Poaceae species, in cold-acclimated and non-acclimated plants under MD and LD. *Deschampsia antarctica* had more Suc content than agricultural Poaceae species (barley, oat, and wheat) and around 2–3 times more Suc content than other wild Poaceae species *(D. caespitosa* and *D. beringensis*) (Fig. 3). The highest amount of Suc was found in *D. antarctica* at the end of the acclimation period under LD and MD conditions (61.5 ± 8.3 mg g^{-1} and 48 ± 12.4 mg g^{-1} dry weight, respectively). Oat had the highest Suc content among the agricultural Poaceae (42.3 ± 0.7 mg g^{-1} dry weight) (Fig. 3). Because *D. antarctica* accumulates mainly Suc we compared Suc/TSS ratio in all Poaceae species at different day lengths in cold-acclimated and non-acclimated plants. *Deschampsia beringensis* had the highest ratio at LD conditions in cold acclimated plants (0.73 ± 0.04). In general, the three *Deschampsia* species showed a higher ratio than the agricultural species (Table I). The highest ratios in agricultural species was 0.24 ± 0.01 for cold acclimated oat at MD conditions and 0.26 ± 0.02 for non-

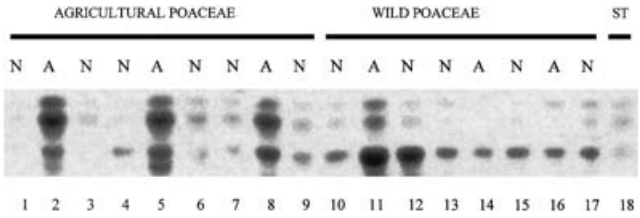

Fig. 4. Sugars present in mature leaf extracts of six species of Poaceae, cold-acclimated (A) and non-acclimated (N) under LD. Non-acclimated plants were kept at 15ºC; plants were cold-acclimated at 4º. F = fructose, G = glucose, S = sucrose. ST = standard of sucrose, fructose and glucose. 1 = non-acclimated wheat at 0 time, 2 = cold-acclimated wheat at 21 days, 3 = non-acclimated wheat at 21 days, 4 = non-acclimated barley at 0 time, 5 = cold-acclimated barley for 21 days, 6 = non-acclimated barley at 21 days, 7 = non-acclimated oat at 0 time, 8 = cold-acclimated oat at 21 days, 9 = non-acclimated oat at 21 days, 10 = non-acclimated *Deschampsia antarctica* at 0 time, 11 = cold-acclimated *D. antarctica*, for 21 days, 12 = non-acclimated *D. antarctica* at 21 days, 13 = non-acclimated *D. caespitosa*, at 0 time, 14 = cold-acclimated *D. caespitosa*, at 21 days, 15 = non-acclimated *D. beringensis*, at 0 time, 16 = cold-acclimated *D. beringensis*, at 21 days, 17 = non-acclimated *D. beringensis* at 21 days, 18 = 3 µl of a mixture of Suc, glucose, fructose standard 0.1 mg ml^{-1}. 1-12 = each line was loaded with 2 µl of concentrated methanolic extract that correspond to 0.02 mg dry weight of leaf tissue.

acclimated wheat at LD. The Suc/TSS ratio in *D. antarctica* increased with day length. Agricultural Poaceae species mainly accumulated glucose and fructose. This pattern of sugars was especially evident in cold-acclimated wheat and barley (Fig. 4).

Dry weight at different day lengths

Plants of *D. antarctica* were separately grown for 21 days at different day lengths (SD, MD and LD), and 15ºC or cold acclimated at 4ºC. Since these experiments were executed at constant photon flux density, radiation integrals were 60, 121 and 159 mol photons m^{-2} seg^{-1}, respectively. The dry masses of shoots and roots were significantly different at LD, when compared to SD and MD (Table II). As expected, dry mass was correlated with radiation integrals at both temperatures (r^2 = 0.81 shoots and r^2 = 0.89 roots), suggesting that more than a photoperiodic effect, LD

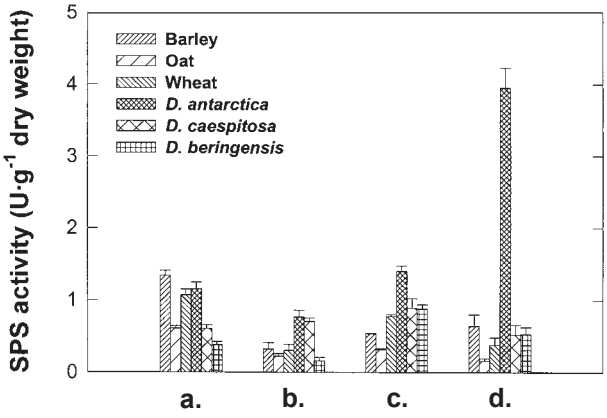

Fig. 5. SPS activity in Poaceae leaves. **a.** non-acclimated at MD = medium day (16/8 h light/dark), **b.** after 21 days of cold acclimation at MD, **c.** non-acclimated after 21 days at LD = long day (21/3 h light/dark), and **d.** after 21 days of cold acclimation at LD. Non-acclimated plants were kept at 15ºC, plants were cold-acclimated at 4º. Values are means ± SE (vertical bars) of three independent experiments.

conditions provided more time for photosynthesis, with a higher radiation integral.

SPS activity

SPS activity was measured in leaves of several agricultural and wild Poaceae species in non-acclimated and cold-acclimated plants at 21 days of MD and LD. We used only MD and LD treatments because they are more similar to the real day length that the leaves of *D. antarctica* plants are exposed during the growing season. The highest activity was found in *D. antarctica* in LD cold-acclimated plants (3.95 ± 0.28 U g^{-1} dry weight), about four times greater than in the other treatments (Fig. 5). Leaf Suc content and SPS activity in *D. antarctica* were highly correlated (r = 0.85 in LD cold-acclimated plants).

Discussion

Deschampsia antarctica grown in the laboratory had a lower content of Suc (6% of dry weight) than plants grown in the field (up to 36% of dry weight) (Zúñiga *et al.* 1996). Suc content in the plants growing in the laboratory,

Table II. *Deschampsia antarctica* ramet dry mass of shoots and roots after 21 days at 15º C or 4ºC and different day lengths. SD = short day (8/16 h light/dark), MD = medium day (16/8 h light/dark) and LD = long day (21/3 h light/dark). Each value corresponds to the mean ± standard error (n = 10). Radiation integral correspond to 21 days of treatment, the values were expressed in mol photons m^{-2} day^{-1}. Different letters represent significant differences between day lengths (P = 0.05).

Day length	Dry mass of roots (mg/ramet) 15ºC	Dry mass of roots (mg/ramet) 4ºC	Dry mass of shoots (mg/ramet) 15ºC	Dry mass of shoots (mg/ramet) 4ºC	Radiation integral (mol m^{-2} day^{-1})
LD	3.86 ± 0.72a	2.20 ± 0.40c	7.18 ± 1.11e	4.10 ± 0.63g	159
MD	1.56 ± 0.36b	0.88 ± 0.20d	4.48 ± 0.65f	2.60 ± 0.37h	121
SD	0.96 ± 0.27b	0.54 ± 0.15d	4.36 ± 0.40 f	2.40 ± 0.22 h	60

however, was at least three times higher than that in agricultural Poaceae species tested, and twice as high as that in wild Poaceae species (*D. beringensis* and *D. caespitosa*). In agricultural Poaceae species, the content of glucose and fructose increased ten to twelve fold during cold acclimation (Zúñiga *et al.* 1998).

Comparing the Suc/TSS ratio among Poaceae species, we observed that, in general, all *Deschampsia* species showed the highest values (Table I). A high proportion of Suc may be important for protection against low temperature during the growing season, when the day length is long at high latitude. It is believed that Suc has an important function in cryoprotection of plants and that this accumulation at low temperature correlates with low temperature tolerance (Linneberger & Steponkus 1980, Newsted *et al.* 1991, Bravo *et al.* 2001). *Deschampsia antarctica* accumulates the highest amount of Suc compared with the other Poaceae species. The differences found in sugar content in Poaceae species may be related to a differential capacity to synthesize and utilize Suc in fructan biosynthesis, as well as a different rate of export to sink organs. *Deschampsia antarctica* accumulates little starch under various day lengths during cold acclimation, allowing the use of photosynthates mainly for Suc and fructans (Bravo *et al.* 2001). Although the flux of triose-P outwards from the chloroplast may regulate Suc biosynthesis, the regulation of this partitioning is not fully understood (Heineke *et al.* 1995).

When plants of *D. antarctica* were transferred for one week to LD (from MD, before initiating the cold acclimation experiment), TSS content increased by 300% in leaves and 200% in crowns. Radiation integrals under LD conditions were 31% higher than that at MD conditions. Thus, this increase in radiation was mainly channelled into sugar accumulation (most probably by synthesis) and not to growth. This hypothesis is supported by the fact that it was possible to find significant differences in dry mass of *D. antarctica* kept at 15 or 4ºC under different photoperiods after only 21 days of treatment (Table II). These results are also consistent with those reported by Xiong *et al.* (2000), who analysed the effect of temperature (7, 12 and 20ºC) on *D. antarctica* plants under an 18/6 h light/dark period. These results indicated that differences in biomass are evident only after 90 days of treatment. The effect of cold was more pronounced in MD plants, with a 300% increase in leaves TSS after 21 days of cold acclimation. This increase is in agreement with the high accumulation of fructans found in MD cold-acclimated plants (Bravo *et al.* 2001). The fructans pools may play an active role in alleviating freezing stress by acting as a reserve from which additional cryoprotective sugars can be made as required (Hurry *et al.* 1994). Increasing the radiation integral, from MD to LD for one week increased sucrose content in leaves and in the crown (160% and 80% respectively). Sucrose content in cold acclimated *D. antarctica* crowns increased in SD (23%), MD (80%) and LD (16%). In winter wheat (*Triticum aestivum* L. cv. Monopol) sucrose content in the crown increases nearly 400%, when plants are kept at high irradiation (800 µmol photons m^{-2} seg^{-1}) and only 260% when plants are cold-acclimated at low radiation (250 µmol photons m^{-2} seg^{-1}) (Savitch *et al.* 2000). These authors suggest that the capacity of winter wheat cultivars to assimilate CO_2 is associated with increased sucrose and fructan biosynthesis in leaves, and fructan biosynthesis in crowns, due to increased sucrose export and increased crown sink capacity for sucrose metabolism, as indicated by increased SST, SS, and acid invertase activity.

Total carbohydrate content, its composition and distribution in plant tissues varies through the year (Suzuki 1989, Turner & Pollock 1998, Yoshida *et al.* 1998). Temperate zone plants may accumulate carbohydrates when sugar synthesis exceeds utilization (Larcher 1995, Murelli *et al.* 1995). Carbohydrate content in roots of wild grasses varies over the year with a maximum in autumn and a minimum in early spring when the plants start to grow (Steen & Larsson 1986). Freezing-tolerant cultivars of winter wheat accumulated high levels of soluble carbohydrates in crowns and leaves at the end of the hardening stage (autumn). These cultivars may respond to sub-zero temperatures and increase hardiness by conversion of fructan to cryoproptective sugars, such as fructose and sucrose (Yoshida *et al.* 1998). Most of the rhizomatous phanerogams on the sub-Antarctic islands appear to store photosynthate in their below-ground organs (Smith 1984). *Parodiochloa flabellata* (=*Poa*) contains no starch but high levels of fructans as storage compounds. Levels were high in the stems (non-photosynthetic leaf sheaths), with 58% (spring) and 73% (autumn) of the dry weight occurring in "middle-aged" leaves; these clearly serve as a principal storage organs (Gunn & Walton 1985). *Poa foliosa* presented maximum carbohydrate levels (up to 28% of dry weight) in the leaf sheaths in late summer (Jenkin 1972). The exact duration of the growing period of *D. antarctica* in the Maritime Antarctic is unknown. Nonetheless, new growth of both roots and leaves commences before the snow cover has melted (Smith 2003). *Deschampsia antarctica* may use the TSS stored in the crown for growth. Later, when day length decreases (close to MD), the plants accumulate carbohydrates in the leaves. This is in agreement with the results of Bravo *et al.* (2001), which show that the highest accumulation of fructans occurred in plants under MD. In the field, when the day length decreases towards our experimental SD (early March), the plants probably store part of the carbohydrates in the crown.

SPS activity increased significantly with day length in *D. antarctica*, but not in the other Poaceae species. The high amount of Suc observed in the leaves was highly and positively correlated with SPS activity in LD plants (r^2 = 0.85). Sucrose accumulation in white clover (*Trifoliun repens*) appears to be associated to changes in photoperiod,

temperature and irradiance (Turner & Pollock 1998). There is a correlation between dry mass of shoots and roots and radiation integral in plants of *D. antarctica* grown at 15ºC and various photoperiods. An increase in temperature also favours growth of this plant (Xiong *et al.* 2000, Bravo *et al.* 2001). Perhaps, during the growing season in the Antarctic, the combined effect of high SPS activity, caused by long days, with concomitant higher temperature and daily radiation integrals, allow rapid growth, assuring its survival. Changes in day length might also be a switch that controls differential accumulation and distribution of carbohydrates in *D. antarctica* plants during the growing season. Further studies on Suc export from the leaves to the crown as well as on fructan accumulation in the crown and the activity of enzymes of fructan synthesis such as Suc fructosyltransferase (SST, EC 2.4.1.99) and fructan:fructan fructosyltransferase (FFT, EC 2.4.1.100) is needed to understand the factors that determine carbohydrate distribution in the grass.

Deschampsia antarctica exhibited higher SPS activity than other Poaceae species. We observed a large increase in SPS activity under long day conditions only in *D. antarctica*. Studies in transgenic tomato, *Arabidopsis* and potato that over-express maize SPS gene have shown increases in fruit and tuber weight (Galtier *et al.* 1995, Signora *et al.* 1998, Tobias *et al.* 1999). Recently, a significantly lower panicle weight than wild-type plants was observed in transgenic rice plants with low SPS activity (Ono *et al.* 1999). Perhaps, a high SPS activity in *D. antarctica* during the growing season leads to rapid growth of vegetative and reproductive structures. Floral phenology of *D. antarctica* has been studied, indicating initiation and preformation in autumn, with the inflorescence overwintering in an early stage of development (Walton 1982). We have observed occasional germination in the inflorescence of *D. antarctica* after a dry period in laboratory growing plants. *Deschampsia antarctica* flower production on Signy Island is greatest in drier habitats, with plants in wet or nitrogen-rich sites often having no inflorescences (Edwards 1974). In contrast to the Arctic flora, true vivipary is unknown in sub-Antarctic plants, although occasionally in wet habitats or during long spells of wet weather *Acaena magellanica*, *Phleum alpinum* and *Poa flabellata* seeds germinated *in situ* in the inflorescence (Smith 1984).

Studies of potato tubers exposed to cold (3–5ºC) showed an increase in the activation state of SPS. The ratio of SPS activity measured under limiting and non-limiting conditions (Vl/Vnl) increased from 10 to 40 %, with the appearance of a novel form of SPS (Deiting *et al.* 1998). Also, spring and winter wheat cultivars exposed to cold temperature presented a differential adjustment of SPS activity. The activation state of SPS increased from 20% to 60% in a cold-acclimated winter wheat cultivar but not in a spring wheat cultivar (Savitch *et al.* 1997). SPS activity, measured under non-limiting conditions in a crude extract of *D. antarctica*, increases nearly 60% when 15ºC plants are changed to LD conditions (Zúñiga *et al.* 1998). Further studies are required to verify if the increase in SPS activity found in *D. antarctica* LD plants could be explained by enzyme activation or by *de novo* synthesis.

Our working hypothesis that long day conditions induce higher SPS activity, leading to higher Suc accumulation has been proved. The highest SPS activity was found under LD conditions and the highest leaf Suc content was found in cold-acclimated plants under LD. SPS activity and leaf Suc content were positively correlated only in LD cold-acclimated leaves (r = 0.85). *Deschampsia antarctica* has the highest Suc content compared with the other Poaceae species analysed. This unusual Suc accumulation and its capacity for rapid growth during the short growing season are some of the features that allow *D. antarctica* to survive the harsh Antarctic condition.

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