

# Changes in soluble carbohydrates in polar Caryophyllaceae and Poaceae plants in response to chilling

Marta Pastorczyk · Irena Gielwanowska ·  
Lesław B. Lahuta

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**Abstract** Four species of flowering plants comprising Arctic populations of *Cerastium alpinum* and *Poa arctica* var. *vivipara* and indigenous Antarctic species *Colobanthus quitensis* and *Deschampsia antarctica* were investigated. Plants derived from natural origins were grown in an experimental greenhouse in Poland (53°47'N and 20°30'E latitude). Plants for experiment were collected during spring of 2010. Soluble carbohydrates in the intact shoots of *C. alpinum* and *C. quitensis*, polar plants of the family Caryophyllaceae, and *D. antarctica* and *P. arctica* var. *vivipara*, representatives of the family Poaceae, were analyzed by gas chromatography, and their involvement in the plants' response to chilling stress was examined. Plant tissues of the examined families growing in a greenhouse conditions (18–20 °C, short day 10/14 h light/darkness) differed in the content and composition of soluble carbohydrates. In addition to common monosaccharides, *myo*-inositol and sucrose, Caryophyllaceae plants contained raffinose family oligosaccharides (RFOs), D-pinitol and mono-galactosyl pinitols. RFOs and D-pinitol were not detected in plants of the family Poaceae which contain 1-kestose, a specific tri-saccharide. The accumulation of significant quantities of sucrose in all investigated plants, RFOs in Caryophyllaceae plants and 1-kestose in Poaceae plants in response to chilling stress

(4 °C for 48 h with a long day photoperiod, 20/4 h) indicates that those compounds participate in the stress response. The common sugar accumulating in cold stress response and probably most important for chilling tolerance of four investigated plants species seems to be sucrose. On the other hand, the accumulation of above-mentioned carbohydrates during chilling stress can be a return to sugars metabolism, occurring in natural environmental conditions. No changes in D-pinitol concentrations were observed in the tissues of *C. alpinum* and *C. quitensis* plants subjected to both low and elevated temperatures, which probably rules out the protective effects of D-pinitol in response to cold stress.

**Keywords** Polar plants · Caryophyllaceae · Poaceae · Carbohydrates · Chilling

## Introduction

Polar plants have developed specific anatomic and physiological traits which enable them to survive in extreme environments (Parnikoza et al. 2011). Resistance to low temperatures, salinity and UV radiation are particularly important determinants of plant survival. In the harsh polar (Antarctic and Arctic) environments, the number of indigenous flowering plants species is very limited. In the Antarctic, only two species grow: *Colobanthus quitensis* (Caryophyllaceae) and *Deschampsia antarctica* (Poaceae). *C. quitensis* and *D. antarctica* have developed unique anatomical structures which enable them to adapt to severe climates, including xerophytic leaves (Romero et al. 1999), structural plasticity of chloroplasts and mitochondria (Gielwanowska 2003; Gielwanowska and Szczuka 2005) as well as organs with highly effective photosynthetic function at low temperatures (Bravo et al. 2007). Survival at sub-zero

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M. Pastorczyk · I. Gielwanowska · L. B. Lahuta (✉)  
Department of Plant Physiology, Genetics and Biotechnology,  
University of Warmia and Mazury in Olsztyn, Oczapowskiego  
1A, 10-719 Olsztyn, Poland  
e-mail: lahuta@uwm.edu.pl

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

temperatures is probably conditioned by the accumulation of protective proteins (Bravo and Griffith 2005; John et al. 2009), fats (Zúñiga et al. 1994), sugars and polyols (Montiel and Cowan 1993; Zúñiga et al. 1996). During acclimatization to freeze stress, *Deschampsia antarctica* tissues accumulate very high amounts of sucrose which reach up to 36 % of dry matter content (Zúñiga et al. 1996). Sucrose is accumulated due to high activity levels of sucrose-6-phosphate synthase (Zúñiga-Feest et al. 2003, 2005), but increased sucrose levels do not protect plant tissues against freezing (Zúñiga-Feest et al. 2009). In *Arabidopsis thaliana* (Klotke et al. 2004), *Medicago sativa* (Cunningham et al. 2003) and *Ajuga reptans* (Peters and Keller 2009), increased amounts of galactinol and raffinose family oligosaccharides (RFOs) are also deposited in vegetative tissues during cold acclimatization. Other compounds which condition cold tolerance (Pontis 1989) include fructans (1-kestose) in *D. antarctica*, and RFOs and D-pinitol in *C. quitensis* plants (Piotrowicz-Cieślak et al. 2005). The spatial configuration of hydroxyl groups in sugar molecules also determines their cryoprotective effects (Turner et al. 2001). Hydroxyl groups have been found to exert protective effects in response to dehydration stress in developing seed embryos of many crop plant species (Peterbauer and Richter 2001; Obendorf and Górecki 2012). The protective role of RFOs and fructans against abiotic stresses was last reviewed by ElSayed et al. (2014) and Van den Ende (2013). However, the predominant objects in studies concerning the role of soluble carbohydrates in abiotic stress tolerance of vegetative tissues are model plants (*Arabidopsis thaliana*, *Thellungiella halophylla*) and crops naturally growing in moderate climate conditions.

The aim of this study was to determine the communities and differences in changes of soluble carbohydrates concentration in the shoots of four polar plant species which are members of the families Caryophyllaceae and Poaceae: *Cerastium alpinum*, *Colobanthus quitensis*, *Deschampsia antarctica* and *Poa arctica* var. *vivipara* in tissues early response to chilling stress. Two of the selected species (*C. quitensis* and *D. antarctica*) are Antarctic indigenous vascular plants. For comparison, *C. alpinum* and *P. antarctica* var. *vivipara* growing in Arctic were used. The opportunity to take those studies was a unique successful propagation of plants deriving from natural polar plants environments (Antarctic and Arctic) in a greenhouse conditions.

## Materials and methods

### Materials

The experiment was performed on four species of flowering plants comprising Arctic populations of *Cerastium alpinum* L. and *Poa arctica* var. *vivipara* R. Br. and

indigenous Antarctic species *Colobanthus quitensis* (Kunth) Bartl. and *Deschampsia antarctica* Desv.

Seeds and live plants were harvested during polar expeditions organized in 2002–2010 in the region of the Henryk Arctowski Polish Antarctic Station on King George Island (South Shetland Islands, 62°09'S and 58°28'W) and the Stanisław Siedlecki Polish Polar Station in Hornsund, Spitsbergen (77°00'N and 15°33'E). Harvested samples were secured and transported to Olsztyn (53°47'N and 20°30'E), Poland.

### Plant cultivation

The harvested specimens were planted, and seeds were sown and grown at 18–20 °C in the greenhouse of the Faculty of Biology and Biotechnology of the University of Warmia and Mazury in Olsztyn (77°00'N and 15°33'E). The established treatments were a continuous source (currently from a year 2002) of vegetatively propagated fresh plant material for laboratory analyses.

### Analyses of plant responses to chilling stress

Plants grown in pots (one plant per 1 dm<sup>3</sup> pot, containing garden soil, in three replications) in May of 2010 were transferred to an incubator (Heraeus, BK6160, Kendro Laboratory Products) at the constant temperature of 20 °C under a 20/4 h photoperiod (light/dark). We selected long-term photoperiod because the growing season in the Antarctic (King George Island, South Shetland Islands) takes just during the Australian summer, when the length of day is 16–20 h. Similarly in the Arctic, the growing season lasts during the long day. After 24 h of adaptation to long day and constant temperature conditions, plants (in two replicates) were subjected to cold stress (4 °C) for 48 h. Shoot samples for analyses of soluble carbohydrates were sampled (from two stems in a duplicate) directly before chilling and 1, 4, 8, 12, 24 and 48 h after the beginning of chilling. After 48 h of chilling, temperature was raised to 20 °C, and the last sample was collected after 24 h. Tissue samples collected at identical time intervals from plants growing at 20 °C served as a control group. The samples were weighed, frozen in liquid nitrogen, stored at –80 °C for 72 h and lyophilized in a freeze dryer. Dried tissues were weighed and pulverized in a ball mill (Retsch MM200) with vibrational frequency of 20 Hz for 2 min. Pulverized tissue samples of 40–50 mg were collected for the extraction of soluble carbohydrates.

Analysis of the content and composition of soluble carbohydrates in vegetative plant tissues by gas chromatography

The extraction of soluble carbohydrates and chromatographic separation were carried out according to the method described

by Lahuta and Górecki (2011). Samples of pulverized tissue (40–50 mg) were combined with 800  $\mu$ l 50 % ethanol and 100  $\mu$ l xylitol (internal standard, concentration of 1 mg/ml). The resulting mixture was heated at 90 °C for 30 min. During heating, the specimens were twice shaken in a microshaker (Vortex Genie 2, 1300 rpm, 2 min). The homogenate was centrifuged (21,000 $\times$ g, 20 min, 4 °C), 400  $\mu$ l of the supernatant was transferred to 1.5 ml Eppendorf tubes filled with 300  $\mu$ l of cationite and anionite mixture (Dowex 50WX8, H<sup>+</sup>, 100–200 mesh; Dowex 2X8, Cl<sup>-</sup>, 50–100 mesh, Fluka) and shaken for 60 min at 1,300 rpm. After centrifugation (21,000 $\times$ g, 10 min, 4 °C), 200  $\mu$ l of the supernatant was transferred to glass chromatography vials and dried until the formation of dry pellet in a vacuum centrifuge (JW Electronic, Polska). Dry pellets for chromatographic analysis were stored on silica gel (Supelco, UK). Directly before chromatographic analysis, carbohydrates were derivatized by adding 200  $\mu$ l of trimethylsilyl imidazole (TMSI) with pyridine (1:1, v/v) and heating the resulting mixture at 80 °C for 1 h. The prepared specimens were analyzed in a gas chromatograph (GC 2010, Shimadzu, Japan) with a flame ionization detector (FID), an autoinjector (AOC-20 s) and an autosampler (AOC-20i). Trimethylsilyl derivatives were separated in the ZEBRON ZB-1 (Phenomenex, USA) capillary column (15 m length, 0.25 mm diameter and 0.1  $\mu$ m thickness of 100 % dimethylpolysiloxane). Samples of 1  $\mu$ l were injected automatically onto the column in the split mode (10:1). The column was heated at 160 to 335 °C at a rate of 20 °C/min, and the final temperature was maintained for 4.25 min. Total separation time was 13.00 min. Helium was the carrier gas. Data were registered with the use of a computer integrator in the CHROMA 3.2 application (Pol-Lab, Poland). Peaks were identified in chromatograms by comparing their retention times with standard retention times. Sugar concentrations were determined based on a simple linear regression model describing the ratio of the surface area of the sugar peak (in the range of 10–200  $\mu$ g) to the surface area of the peak produced by 100  $\mu$ g of the internal standard (xylitol).

#### Statistical analysis

The results of carbohydrates determination were subjected to analysis of variance (ANOVA) and Newman–Keuls post test ( $P < 0.001$ ).

## Results

### Content and composition of soluble carbohydrates in the shoots of *Cerastium alpinum* and *Colobanthus quitensis* control plants

The qualitative composition of soluble carbohydrates in the vegetative tissues of *Cerastium alpinum* and *Colobanthus*

*quitensis* was nearly identical before chilling. The extracts contained fructose, glucose, sucrose, galactinol, RFOs—raffinose, stachyose and verbascose, cyclitols (*myo*-inositol, *D*-ononitol and *D*-pinitol) and their  $\alpha$ -*D*-galactosides (galactinol, galactoononitol and galactopinitol, respectively).

The monosaccharide (fructose, glucose) concentration in the analyzed plant tissues was relatively low, ranging from 0.3 % of dry weight (DW) in *C. alpinum* to 0.4 % DW in *C. quitensis*, whereas sucrose (0.7 % DW) and RFO levels (1.3–2.3 % DW, Table 1) were considerably higher. RFOs accounted for 21–23 % of total soluble carbohydrates (SCs). Stachyose was the predominant compound in the RFOs fraction. The analyzed tissues contained *myo*-inositol and its methyl derivatives: *D*-ononitol and *D*-pinitol. Ononitol concentrations were low (data not presented), and *D*-pinitol was the predominant compound with a 38–40 % share of the SCs. *C. alpinum* also contained small amounts of *D*-pinitol mono-galactosides and di-galactosides (galactopinitol A—GPA, galactopinitol B—GPB, di-galactopinitol A—ciceritol). GPA was the only *D*-pinitol  $\alpha$ -*D*-galactoside in *C. quitensis*, but it was noted in trace quantities (<0.1 % DW).

Extracts of *C. alpinum* and *C. quitensis* tissues contained also compounds which could not be identified based on the available standards. The first compound was characterized by retention time of  $t_R = 8.65$  (UNK 1), which could be indicative of an oligosaccharide with the degree of polymerization (DP) of 3. The remaining three peaks (between stachyose and verbascose,  $t_R = 10.85$ ,  $t_R = 11.02$ ,  $t_R = 11.89$ ) can probably be attributed to oligosaccharides with the DP of 4. The content of the above compounds was calculated based on simple linear regression models of raffinose (UNK 1) and stachyose (UNKs 2–4), respectively.

### Changes in the concentration of soluble carbohydrates in response to chilling stress

The incubation of *C. alpinum* and *C. quitensis* plants in a controlled environment chamber at 20 °C and under a 20/4 h photoperiod for 48 h (*C. alpinum*) or 8 h (*C. quitensis*) gradually decreased the concentrations of soluble carbohydrates. Cold stress induced SCs accumulation in *C. alpinum* (Table 2; Fig. 1a, c) and inhibited carbohydrate depletion in *C. quitensis* (Table 2; Fig. 1b, d).

Sucrose accumulation was reported already after 4–8 h of chilling. Sucrose levels increased for 24 h in *C. alpinum*, but prolonged chilling followed by heating lowered sucrose concentrations (Fig. 1a). The increase in sucrose levels in response to cold stress was not accompanied by the relevant changes in monosaccharide concentrations. During incubation at 20 °C, RFOs were successively depleted, but under exposure to low temperatures this process was

**Table 1** The concentration of soluble carbohydrates in shoots of Caryophyllaceae (*Cerastium alpinum* and *Colobanthus quitensis*) and Poaceae (*Deschampsia antarctica* and *Poa arctica*)

| Soluble carbohydrates             | <i>Cerastium alpinum</i><br>(mg g <sup>-1</sup> DW) | <i>Colobanthus quitensis</i><br>(mg g <sup>-1</sup> DW) | <i>Deschampsia antarctica</i><br>(mg g <sup>-1</sup> DW) | <i>Poa arctica</i> var.<br><i>vivipara</i><br>(mg g <sup>-1</sup> DW) |
|-----------------------------------|---|---|--|---|
| Fructose                          | 0.69 ± 0.11   | 1.49 ± 0.42   | 11.81 ± 3.05   | 14.14 ± 1.28  |
| Glucose                           | 1.55 ± 0.15   | 2.55 ± 0.57   | 17.86 ± 1.42   | 12.15 ± 1.22  |
| Galactose                         | 0.93 ± 0.08   | 0.31 ± 0.05   | 0.12 ± 0.01  | 0.16 ± 0.01   |
| Sorbitol                          | –   | –   | 0.79 ± 0.33  | 1.04 ± 1.12   |
| <i>myo</i> -Inositol              | 0.63 ± 0.02   | 1.8 ± 0.26  | 0.72 ± 0.11  | 0.27 ± 0.03   |
| Sucrose                           | 6.90 ± 0.48   | 7.22 ± 0.97   | 49.57 ± 3.86   | 18.43 ± 4.41  |
| Maltose                           | –   | –   | 0.73 ± 0.05  | 0.63 ± 0.12   |
| Galactinol                        | 0.1 ± 0.01  | 0.07 ± 0.01   | 0.01 ± 0.00  | 0.2 ± 0.04  |
| 1-Kestose                         | –   | –   | 22.07 ± 3.12   | 3.53 ± 0.06   |
| Total RFOs                        | 23.01 ± 0.8   | 13.11 ± 3.03  | –  | –   |
| Raffinose                         | 6.36 ± 0.36   | 2.16 ± 0.07   | –  | –   |
| Stachyose                         | 14.17 ± 0.62  | 9.22 ± 2.26   | –  | –   |
| Verbascose                        | 2.48 ± 0.63   | 1.74 ± 0.21   | –  | –   |
| D-Pinitol                         | 43.17 ± 3.33  | 20.27 ± 2.51  | –  | –   |
| Total galactopinitols             | 2.38 ± 0.1  | 0.13 ± 0.02   | –  | –   |
| GPA                               | 1.23 ± 0.05   | 0.13 ± 0.02   | –  | –   |
| GPB                               | 0.74 ± 0.05   | –   | –  | –   |
| Ciceritol                         | 0.41 ± 0.01   | –   | –  | –   |
| D-Ononitol                        | 0.55 ± 0.03   | 2.22 ± 0.27   | –  | –   |
| Total unknown (DP3, DP4, DP5)     | 24.91 <sup>a</sup> ± 3.27                           | 7.01 <sup>a</sup> ± 3.27                                | 6.41 <sup>b</sup> ± 1.26                                 | 1.56 <sup>b</sup> ± 0.32  |
| Total soluble carbohydrates (SCs) | 105.33 ± 3.51                                       | 56.13 ± 5.20  | 107.97 ± 1.66  | 55.20 ± 1.96  |

Means ( $n = 3$ ) ± SE<sup>a</sup> DP3, DP4; <sup>b</sup> DP3**Table 2** Changes in the concentration of total soluble carbohydrates in shoots of Caryophyllaceae (*Cerastium alpinum* and *Colobanthus quitensis*) and Poaceae (*Deschampsia antarctica* and *Poa arctica*) plants growing at 20 °C (control) or 4 °C (chilling stress)

| Treatment<br>(h) | <i>Cerastium alpinum</i> |                     | <i>Colobanthus quitensis</i> |                     | <i>Deschampsia antarctica</i> |                      | <i>Poa arctica</i>  |                     |
|------------------|--------------------------|---------------------|------------------------------|---------------------|-------------------------------|----------------------|---------------------|---------------------|
|                  | mg g <sup>-1</sup> DW    |                     |                              |                     |                               |                      |                     |                     |
|                  | 20 °C                    | 4 °C                | 20 °C                        | 4 °C                | 20 °C                         | 4 °C                 | 20 °C               | 4 °C                |
| 0                | 105.33 <sup>a</sup>      | –                   | 56.13 <sup>a</sup>           | –                   | 107.97 <sup>a</sup>           | –                    | 55.20 <sup>a</sup>  | –                   |
| 1                | 101.37 <sup>a</sup>      | 105.06 <sup>a</sup> | 56.99 <sup>a</sup>           | 60.13 <sup>a</sup>  | 102.82 <sup>a</sup>           | 100.78 <sup>a</sup>  | 67.60 <sup>ab</sup> | 74.23 <sup>ab</sup> |
| 4                | 87.54 <sup>a</sup>       | 101.43 <sup>a</sup> | 60.34 <sup>a</sup>           | 64.99 <sup>ac</sup> | 130.81 <sup>ab</sup>          | 137.89 <sup>ab</sup> | 55.42 <sup>a</sup>  | 77.83 <sup>b</sup>  |
| 8                | 90.49 <sup>ab</sup>      | 142.42 <sup>d</sup> | 47.48 <sup>ab</sup>          | 63.53 <sup>ad</sup> | 155.06 <sup>c</sup>           | 109.25 <sup>a</sup>  | 40.39 <sup>a</sup>  | 82.72 <sup>b</sup>  |
| 12               | 74.09 <sup>b</sup>       | 149.41 <sup>d</sup> | –*                           | –*                  | 78.44 <sup>ad</sup>           | 152.38 <sup>c</sup>  | 52.22 <sup>ac</sup> | 71.39 <sup>ab</sup> |
| 24               | 91.26 <sup>ab</sup>      | 162.60 <sup>e</sup> | –*                           | –*                  | 89.40 <sup>ad</sup>           | 158.85 <sup>c</sup>  | 51.48 <sup>ac</sup> | 62.88 <sup>ab</sup> |
| 48               | 47.24 <sup>c</sup>       | 130.40 <sup>d</sup> | –*                           | –*                  | 114.04 <sup>ad</sup>          | 139.45 <sup>bc</sup> | –*                  | 92.81 <sup>d</sup>  |
| 72*              | 73.46 <sup>b</sup>       | 162.63 <sup>e</sup> | –*                           | –*                  | 55.91 <sup>d</sup>            | 155.43 <sup>c</sup>  | 52.77 <sup>ac</sup> | 95.48 <sup>d</sup>  |

Means ( $n = 3$ ). Values with different superscripts (a–e) are significantly different ( $p < 0.05$ )

72\* after 24 h of recovery, –\* not measured due to insufficient amount of plant material

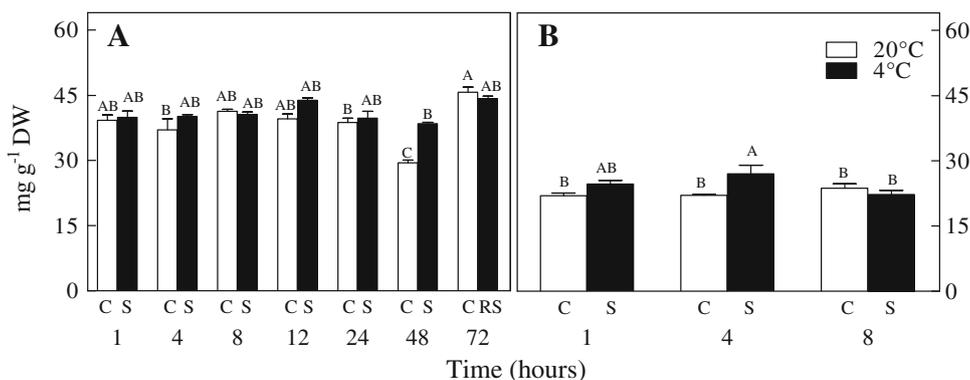
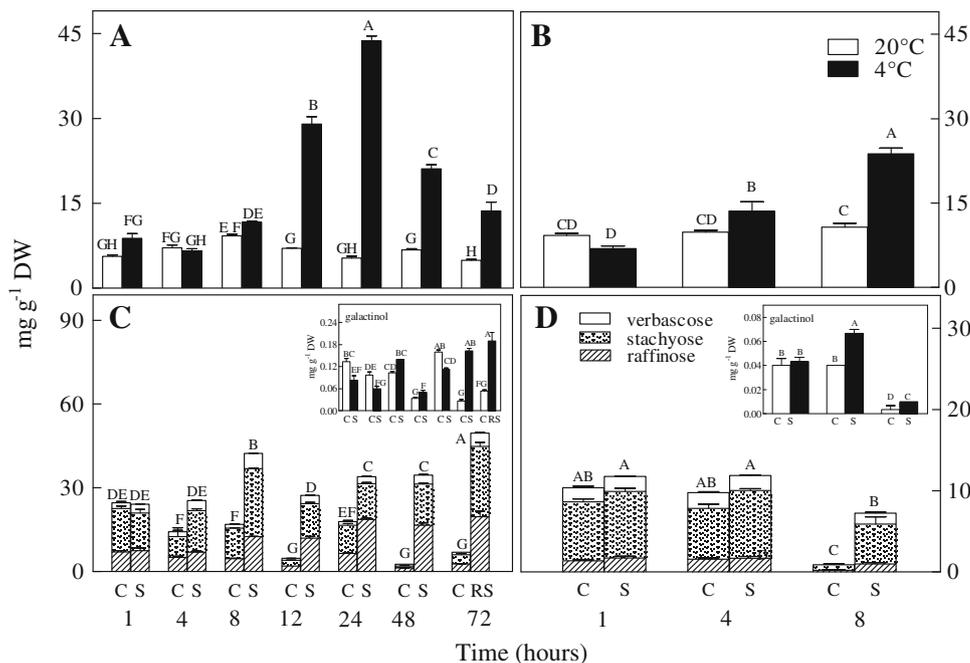
slowed down (Fig. 1d), and it was reversed in *C. alpinum* (Fig. 1c) where the accumulation of RFOs increased. In *C. alpinum*, RFOs levels continued to increase during successive 24 h of heating (Fig. 1c).

No significant variations in the concentration of D-pinitol, the main SCs component, were observed in the analyzed plant species (Fig. 2). Pinitol levels remained relatively stable in the tissues of both control and chilled plants.

Analysis of the composition and concentration of soluble carbohydrates in *Deschampsia antarctica* and *Poa arctica* var. *vivipara* shoots

*Deschampsia antarctica* and *Poa arctica* var. *vivipara* shoots had a different composition of the SCs fraction than *Cerastium alpinum* and *Colobanthus quitensis* (Table 1). In addition to *myo*-inositol and sucrose, simple sugars which

**Fig. 1** Changes in the concentration of sucrose (a and b), raffinose family oligosaccharides (c and d), and galactinol (boxes inside c and d) in *Cerastium alpinum* (a and c) and *Colobanthus quitensis* (b and d) during chilling stress (up to 8–48 h) and after 24 h of recovery (a and c). C control (20 °C), S cold stress (4 °C), RS recovery after cold stress (20 °C). Values are means of three replicates  $\pm$  SE. The same letters above the bars indicate no significant ( $p < 0.01$ ) differences after Newman–Keuls test



**Fig. 2** Changes in the concentration of D-pinitol in *Cerastium alpinum* (a) and *Colobanthus quitensis* (b) during chilling stress (up to 8–48 h) and after 24 h of recovery (a). C control (20 °C), S cold

stress (4 °C), RS recovery after cold stress (20 °C). Values are means of three replicates  $\pm$  SE. The same letters above the bars indicate no significant ( $p < 0.01$ ) differences after Newman–Keuls test

occur commonly in the studied species of both plant families, the tissues of *D. antarctica* and *P. arctica* var. *vivipara* contained fructan (1-kestose) and sorbitol (Table 1), whereas D-pinitol, D-ononitol and RFOs, compounds characteristic of Caryophyllaceae, were not found.

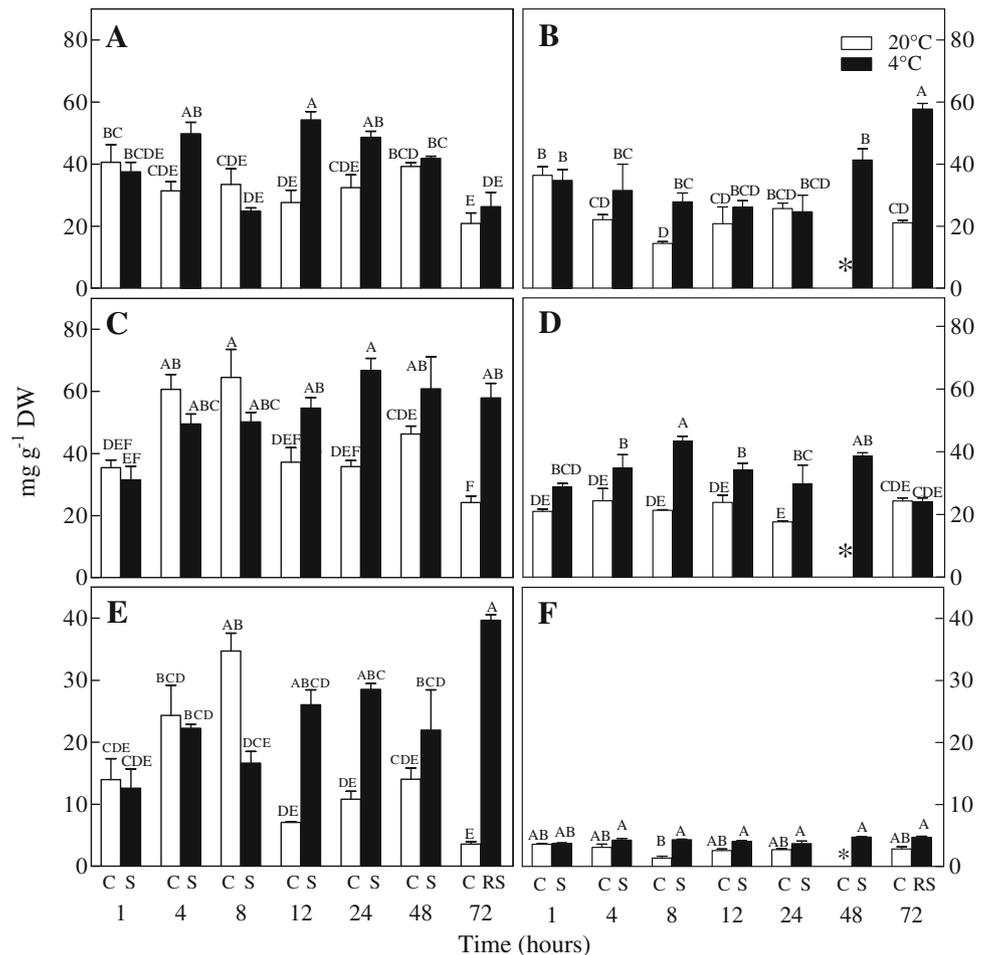
Sucrose was the predominant SCs compound in both species, and it accounted for nearly half (45.91 %) of all soluble carbohydrates in *D. antarctica*. Both *D. antarctica* and *P. arctica* var. *vivipara* were characterized by significant concentrations of monosaccharides: fructose, glucose and small quantities of galactose. In the tissue extracts of *P. arctica* var. *vivipara*, fructose and glucose had more than a 47 % combined share of all SCs. The vegetative tissues of *D. antarctica* contained high amounts of 1-kestose (2.2 % DW), whereas significantly lower levels of this compound (0.35 % DW) were reported in *P. arctica* var. *vivipara*.

Galactinol was the only galactosyl cyclitol which was detected in insignificant quantities. Both RFOs and isomers or methyl derivatives of *myo*-inositol were not found. In chromatograms, 1-kestose was followed by an unknown compound (UNK 1) which could not be identified based on the available standards. Its retention time ( $t_R = 9.72$ ) could be indicative of an oligosaccharide with the DP of 3. The content of the unknown compound was determined based on a simple linear regression model for 1-kestose.

Changes in the concentration of soluble carbohydrates in *Deschampsia antarctica* and *Poa arctica* var. *vivipara* shoots in response to cold stress

The incubation of *Deschampsia antarctica* and *Poa arctica* var. *vivipara* plants at 20 °C and under a 20/4 h

**Fig. 3** Changes in the concentration of monosaccharides (a, b), sucrose (c, d) and 1-kestose (e, f) in *Deschampsia antarctica* (a, c, e) and *Poa arctica* (b, d, f) during cold stress and after 24 h of recovery. C control (20 °C), S cold stress (4 °C), RS recovery after cold stress (20 °C). Values are means of three replicates  $\pm$  SE. The same letters above the bars indicate no significant ( $p < 0.01$ ) differences after Newman-Keuls test



photoperiod for 48 h (control conditions) induced different changes in their SCs profiles (Table 2). In both species, changes were observed in two phases covering early (1–8 h) and late responses (12–24/48 h). In the first phase, the content of sucrose and 1-kestose in *D. antarctica* increased rapidly (Fig. 3c, e), but remained unchanged in *P. arctica* var. *vivipara* (Fig. 3d, f). In *P. arctica* var. *vivipara* the level of monosaccharides decreased (Fig. 3b). Between hours 8 and 12 of incubation, the levels of sucrose and 1-kestose decreased in *D. antarctica*, whereas an estimated 20 % increase in monosaccharides was observed in *P. arctica* var. *vivipara*. SCs concentrations increased in *D. antarctica* between hours 12 and 48 and decreased again after the next 24 h (Fig. 3a, c, e). In *P. arctica* var. *vivipara* no similar rhythmic changes in SCs content occurred (Fig. 3b, d, f).

Chilling affected the observed rhythm of changes in SCs levels by stimulating carbohydrate accumulation which was maintained after successive 24 h of heating. The rhythm of changes in SCs concentrations was correlated with the accumulation/decomposition of the predominant

carbohydrates—sucrose, glucose, fructose (Fig. 3a–d) and 1-kestose (Fig. 3e, f).

## Discussion

The presence of sucrose, RFOs and cyclitols was determined in the vegetative tissues of Antarctic flowering plants by Zúñiga et al. (1996) and Piotrowicz-Cieślak et al. (2005). The accumulation of the above SCs could be a mechanism which conditions plant resistance to cold stress (Bravo et al. 2001; Krasensky and Jonak 2012). In our study, all of the analyzed plant species responded to chilling by accumulating sucrose (Figs. 1 and 3). Our findings and data from literature (Guy et al. 2008) confirm that sucrose, an osmotically active compound, participates in the cold stress response. This study analyzed early and short-term responses to chilling stress (up to 48 h), but intensified sucrose accumulation was also observed in plants subjected to chilling and freezing for several weeks (Zúñiga-Feest et al. 2009). However, the accumulation of

sucrose did not condition frost resistance in the Antarctic species of *Deschampsia antarctica* and *Colobanthus quitensis* (Zúñiga-Feest et al. 2009). In *C. alpinum* the concentration of sucrose decreased between 24 and 48 h of chilling stress, reaching level as low as after 24 h of recovery (Fig. 1c). It can be speculated that the rate of recovery is more rapid in this species as compared to plants belonging to Poaceae (Fig. 3c, d). The accumulation of sucrose in early response to chilling stress was also found in tissues of *Haberlea rhodopensis*, a resurrection species with extreme resistance to desiccation, stress-sensitive *Arabidopsis thaliana* and stress-tolerant *Thellungiella halophylla* (Benina et al. 2013). However, the accumulation of sucrose was transient in *H. rhodopensis* and *A. thaliana* but sustained in *T. halophylla* after the return to optimal temperature (Benina et al. 2013). The increasing concentration of sucrose can be a result of decreasing accumulation of transitory starch in chloroplasts under low temperature conditions (Usadel et al. 2008; Purdy et al. 2013). In *Arabidopsis* cold stress induced rapid (during the first 6 h after transfer of plants from 20 to 4 °C) accumulation of sucrose and monosaccharides—glucose and fructose (Usadel et al. 2008). Carbon partitioning toward sucrose accumulation can be a result of both limitation of phloem export and inhibition of starch synthesis due to accumulation of phosphorylated intermediates (Usadel et al. 2008). Sucrose biosynthesis at low temperature in the *Arabidopsis* plants over-expressing sucrose phosphate synthase reduced the inhibition of photosynthesis, maintained the mobilization of carbohydrates from source leaves to sinks and increased the rate at which freezing tolerance developed (Strand et al. 2003; Lundmark et al. 2006). Cold stress combined with high light intensity triggers photoinhibition of the chloroplast, affects integrity of plasma membrane (Sandve et al. 2011) and ultrastructure of different organelles, especially chloroplasts (Kratsch and Wise 2000). It is possible that sucrose, which is able to decrease the lateral mobility of phospholipids in the fully rehydrated, liquid crystalline membrane (Van den Bogaart et al. 2007) can prevent also decrease in membrane fluidity occurring at 0–4 °C (Somerville and Browse 1996). Thus, the protective role of sucrose could be explained not only by their role as an osmoprotectant (in cytosol and vacuole) but also as a compatible solute, protecting structure of membranes. Recently, a concept is emerging in which sugars such as sucrose, RFOs and fructans, known to contribute indirectly to classic antioxidative mechanisms, are also involved in direct reactive oxygen species (ROS) quenching in different organelles, thereby contributing to abiotic stress tolerance (Keunen et al. 2013). However, the role of sucrose as a direct ROS scavenger remains to be proved (Van den Ende and Valluru 2009).

Our study was the first to demonstrate that an increase in the sucrose content of *C. alpinum* was accompanied by a successive accumulation of RFOs (Fig. 1). The results of experiments investigating *Ajuga reptans* (Bachmann et al. 1994; Peters and Keller 2009), *Medicago sativa* (Cunningham et al. 2003) and *Arabidopsis* (Klotke et al. 2004; Zuther et al. 2004; Usadel et al. 2008; Davey et al. 2009) suggest that RFOs participate in vegetative tissue responses to chilling and freezing. The presence of or changes in transcript levels of genes encoding enzymes responsible for galactinol and RFOs biosynthesis could also indicate that RFOs are involved in the abiotic stress response (Guy et al. 2008). In *Arabidopsis*, abiotic stress (drought, cold) induced the expression of various isoforms of galactinol synthase genes (*Gols*), an enzyme which catalyzes the first stage of the RFOs biosynthesis pathway involving in the synthesis of galactinol from UDP-galactose and *myo*-inositol. The resulting galactinol serves as a key donor of galactose residues in successive synthesis reactions of raffinose and its higher homologues (Obendorf and Górecki 2012). Three out of the seven identified *Gols* genes in *Arabidopsis*: *AtGols1*, *AtGols2*, *AtGols3* are activated in response to abiotic stresses. In plants exposed to low temperatures, only one gene, *AtGols3*, was activated (Taji et al. 2002). An increase in transcription levels of *Gols* was observed in the leaves of tomato seedlings exposed to cold stress (Downie et al. 2003). Increased enzyme activity levels and the accumulation of galactinol and raffinose were also reported in vetch (*Vicia villosa* Roth.) seedlings subjected to osmotic stress (Lahuta and Górecki 2011).

At low temperatures, raffinose is accumulated in the leaf chloroplasts of the bugle (*Ajuga reptans* L.), spinach (*Spinacia oleracea* L.) and thale cress (*Arabidopsis thaliana* (L.) Heynh.) (Schneider and Keller 2009), which could play a key role in the acquisition of cold tolerance. In *Arabidopsis*, the expression of stachyose synthase from *Vigna angularis* leads to the synthesis and accumulation of stachyose in the cytosol at low temperatures, which however does not increase the freezing tolerance of leaves (Iftime et al. 2011). The protective effects of raffinose and/or stachyose on galactolipids in thylakoid membranes have not been confirmed (Shimajima and Ohta 2011).

In the tissues of *D. antarctica* exposed to chilling stress, changes in SCs concentrations were correlated with changes in the levels of sucrose and 1-kestose (Fig. 3C, E). After sucrose, 1-kestose was the second most abundant compound in the vegetative tissues of *D. antarctica*, and the profile of its changes corresponded to changes in total SCs levels. In *D. antarctica* increasing sucrose-6-phosphate synthase (SPS) activity and sucrose content in leaves were positively correlated only in long days cold acclimated plants (Zúñiga-Feest et al. 2003). Shoots of *Poa arctica* var. *vivipara* contained significantly lower amounts

of 1-kestose. The accumulation of 1-kestose under stress could be a defense response to low temperatures, but it was demonstrated only in studies of grasses exposed to drought (Amiard et al. 2003) and cold (Pontis 1989; Del Viso et al. 2011). Kestose belonging to the inulin-type fructans can protect structure of membrane under abiotic stresses (Valluru and Van den Ende 2008).

Cyclitols are osmotically active compounds which also increase plant resistance to salt stress, low temperatures and depletion of soil moisture (Yancey 2005). *Myo*-inositol is the most common cyclitol in the cytoplasm of plant and animal cells and in organelles with one and two membranes (Lackey et al. 2003). *Myo*-inositol forms isomers, such as *D-chiro*-inositol, and methyl derivatives, including *D*-pinitol and *D*-ononitol. Whereas *myo*-inositol is commonly found in the cells of various organisms, its isomers and methyl derivatives were detected only in selected plant species, which could have chemotaxonomic implications (Monson et al. 2006). The analyses of the content and composition of SCs in the tissues of *Cerastium alpinum* and *Colobanthus quitensis* revealed high cyclitols level with a predominance of *D*-pinitol. Pinitol levels were relatively stable in the tissues of control and chilled plants (Fig. 2). It could be assumed that *D*-pinitol does not participate directly in cold stress responses. Small quantities of mono-galactosides of *D*-pinitol were detected in *C. alpinum* and *C. quitensis*. However, the levels of those compounds were stable during tissues chilling (data not shown). High cyclitols concentration accounting for around 50 % of total SCs were observed in four plant species of the family Caryophyllaceae which are commonly found in the alpine zone of the Rocky Mountains (Colorado, USA). Cyclitol levels remain constant throughout the entire growing season, and they are regarded as a constant component of SCs which induce tolerance to drought through osmotic protection (Monson et al. 2006).

In this study of four species of polar plants exposed to short-term cold stress (4 °C, 48 h), sucrose was universally accumulated in early stages of the response to low temperatures. Our study was the first to demonstrate that chilling stress induces the RFOs biosynthesis pathway in plants of the family Caryophyllaceae and the 1-kestose biosynthesis pathway in Poaceae plants. Pinitol concentrations in the tissues of Caryophyllaceae plants remained constant, which suggests that this metabolite does not participate in the early cold stress response.

**Author contribution** Ph.D. Marta Pastorczyk—experimental part of the study: plants cultivation, samples collection and preparation for analysis, analyses of soluble carbohydrates by gas chromatography method; statistical analysis of results; manuscript draw (figures and tables, references, main text). Ph.D. Irena Gielwanowska—the concept of the study and experiment design, discussion of

results, manuscript preparation. Prof. L. Lahuta—revision of the results of gas chromatography analyses, results presentation design, discussion of results.

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