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The effect of sodium fluoride on seeds germination and morphophysiological changes in the seedlings of the Antarctic species *Colobanthus quitensis* (Kunth) Bartl. and the Subantarctic species *Colobanthus apetalus* (Labill.) Druce

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Abstract: The phytotoxic effects of fluoride and its derivatives on the seeds and seedlings of the *Colobanthus apetalus* and *Colobanthus quitensis* were studied. This is a first study to evaluate the influence of sodium fluoride (NaF) on the morphophysiological and biochemical processes on two *Colobanthus* species. The influence of various concentrations of NaF (9 mM, 19 mM, 29 mM) on the germination capacity and germination rate of seeds, seedlings growth and the proline content of plant tissues was analyzed under laboratory conditions (20/10°C, 12/12 h). The seeds of *C. apetalus* were collected from a greenhouse, whereas the seeds of *C. quitensis* were collected in Antarctica and in a greenhouse (Olsztyn, Poland). The tested concentrations of NaF did not suppress the germination of *C. apetalus* seeds, but the germination of *C. quitensis* seeds was inhibited. Sodium fluoride mainly inhibited root growth of *C. apetalus* and *C. quitensis*. In both analyzed species, the free proline content of seedlings increased significantly under exposure to NaF. The results of this study clearly indicate that *C. apetalus* and *C. quitensis* are highly resistant to NaF stress.

Key words: Antarctic, *Colobanthus apetalus*, *Colobanthus quitensis*, fluoride stress.

Introduction

Fluorine (F) is the most abundant halogen on the Earth, but fluorinated secondary metabolites are rare in nature (O'Hagan and Harper 1999). It is believed that F is not an essential element for plants. Fluorine and its compounds are the most phytotoxic pollutants in air and soil (Weinstein and Davison 2004) which inhibit seed germination and plant growth, cause tissue necrosis and leaf chlorosis, inhibit photosynthesis and respiration (Fornasiero 2001; Gadi *et al*. 2012).

The origin of fluorides in Antarctica. — Volcanoes are a major natural source of aerosol particles containing F compounds, mainly hydrogen fluoride, which can condense onto ash and tephra particles in the volcanic plume. For example, Mount Erebus, currently the most active volcano in Antarctica, emits a variety of chemical species, including significant quantities of hydrogen fluoride (9.2 Gg/year in 1991) (Ilyinskaya *et al*. 2010). If hydrogen fluoride was be distributed uniformly over Antarctica's area of 14×10^6 km^2 and deposited in snow, it would contribute 0.62 kg km^{-2} year^{-1} of F (Zreda-Gostynska and Kyle 1997).

Bird guano and feathers are the main sources of biogenic elements for Antarctic tundra communities (Rakusa-Suszczewski and Sierakowski 1993). The breeding activities of maritime birds, especially the dominant three species of *Pygoscelis* penguines, have contributed to the accumulation of ornithogenic soils in many coastal regions of Antarctica. The guano of penguins has a unique composition which is determined by the penguin diet. A six-year study revealed that Adèlie penguins diets were composed of 95.4% krill (*Euphausia superba*), 1.6% fish and 3% other prey (Trivelpiece *et al*. 1990). Fluorine content is determined at 0.11% in krill and 2.3% in penguin guano, and F deposition in penguin breeding sites is estimated at 43 g per m^2/year (Tatur 1987).

Plant growth in Antarctica. — Antarctica and Southern Ocean Islands are characterized by severe weather conditions such as water deficit or low temperature, and they are inhabited by relatively few plant species (Alberdi *et al*. 2002; Giełwanowska *et al*. 2011). These include native species of flowering plants, among them *Deschampsia antarctica* Desv. (Poaceae) grass, *Colobanthus quitensis* (Kunth) Bartl. (Caryophyllaceae) (Giełwanowska *et al*. 2005; Parnikoza *et al*. 2011; Androsiuk *et al*. 2015), as well as several invasive species such as *Poa annua* L. (Poaceae) (Olech and Chwedorzewska 2011), and *Cerastium fontanum* Baumg. and *Sagina procumbens* L. rom Caryophyllaceae family (Convey *et al*. 2006). Polar flowering plants have developed structural and functional adaptive mechanisms which enable them to grow and develop in extreme environments (Giełwanowska and Kellmann-Sopyła 2015; Kellmann-

Sopyła *et al*. 2015; Kellmann-Sopyła and Giełwanowska 2015). Adaptive changes have been widely studied in *Colobanthus quitensis*, a native species of the Antarctic geobotanical zone with distribution on South America, South Georgia, the Antarctic Peninsula and South Shetland Islands (West 1991; West and Cowley 1996). Morphophysiological changes in *C. quitensis* have been described in both vegetative (Ruhland and Day 2000; Xiong *et al*. 2002; Bascuñán-Godoy *et al*. 2006; Bravo *et al*. 2007; Bascuñán-Godoy *et al*. 2010; Giełwanowska *et al*. 2014; Pastorczyk *et al*. 2014) and generative tissues (Giełwanowska *et al*. 2011; Kellman-Sopyła and Giełwanowska 2015; Kellman-Sopyła *et al*. 2015; Kellman-Sopyła *et al*. 2017). However, these mechanisms have never been analyzed in *Colobanthus apetalus* (Labill.) Druce, a subpolar species which is also found in South America, mainland Australia and Tasmania, New Zealand and several Southern Ocean Islands, including Subantarctic Macquarie Island (West and Cowley 1996).

Previous studies on Antarctic and Subantarctic flowering plants focused mainly on specific mechanisms of adaptation to extreme and variable environmental conditions (Bascuñán-Godoy *et al*. 2006; Bravo *et al*. 2007; Giełwanowska *et al*. 2011; Giełwanowska *et al*. 2014; Pastorczyk *et al*. 2014; Kellmann-Sopyła *et al*. 2015). However, it is not known how chemical stress, such as exposure to sodium fluoride (NaF), influence on physiological processes in *C. apetalus* and *C. quitensis*. Currently, Tatur *et al*. (1997) only reported that abandoned penguin rookeries are abundant in highly available nutrients and create a favorable environment for tundra development. Thus, we examined the hypothesis that *C. apetalus* and *C. quitensis* are highly resistant to NaF and develop morphophysiological and biochemical strategies to alleviate toxic effects of NaF. To confirm our hypothesis we used a high concentration of NaF and examined the influence of this chemical stressor on germination capacity of seeds and some important morphophysiological and biochemical features of *C. apetalus* and *C. quitensis* seedlings. Performed comparative analyses on two *Colobanthus* species may explain how plants tolerate NaF stress. The results will expand our knowledge about the responses of subpolar and polar plants of the genus *Colobanthus* to chemical stress.

Materials and methods

Plant materials. — The study was performed on two species of the genus *Colobanthus*: *Colobanthus apetalus* and *Colobanthus quitensis*. Seeds of *C. apetalus* were collected from South-East shore of Lago Roca, Tierra del Fuego National Park, in the vicinity of Ushuaia (54°50'S 68°30'W; Argentina, Subantarctic). Seeds of *C. quitensis* were collected from Lion's Rump, King George Bay (62°08'S, 58°08'W; King George Island, West Antarctica). The

collected seeds were germinated in December 2013, and seedlings were planted in a greenhouse of the University of Warmia and Mazury in Olsztyn (53°47'N, 20°30'E; Poland, Europe) in January 2014. Plants in greenhouse are grown at a temperature of 20°C and 16/8 h photoperiod, in pots filled with a 1:1:1 mixture of hortisol, sand and peat. The studies used seeds of *C. apetalus* and *C. quitensis* which were collected from greenhouse-grown plants in the summer of 2015 (June-September). In the studies also seeds of *C. quitensis* harvested in their natural habitats in Antarctica in March 2012 were used.

Germination of *C. apetalus* and *C. quitensis* seeds under exposure to stress. — The germination capacity of *C. apetalus* and *C. quitensis* seeds was analyzed under exposure to sodium fluoride. The seeds of both species were incubated on Petri dishes lined with filter paper soaked with pure water as control and 5 ml of aqueous solution of NaF at concentrations of 9 mM, 19 mM and 29 mM. Every tested variant was analyzed in 4 replications of 9 *C. apetalus* seeds (due to a limited number of seeds) and 25 *C. quitensis* seeds each. The total number of *C. apetalus* seeds used in germination tests was 144 and for *C. quitensis* was 400. The seeds were incubated in environmental chambers at a temperature of 20/10°C and 12/12 h photoperiod. Petri dishes were wrapped in plastic film to prevent water evaporation and changes in NaF concentration. After 15 days non-germinated seeds were transferred to new Petri dishes and germinated as above. The number of germinated seeds was counted daily for 30 days. After this time the final germination percentage (%) was expressed as:

$$G\ (\%) = (A/B) \times 100$$

where, A is the total number of seeds germinated and B is the total number of seeds tested. Mean germination time (Pieper's index) was calculated according to the below formula:

$$\text{Pieper's index} = \frac{\sum(n \cdot t)}{N}$$

where n is a number of germinated seeds on a given day, t is a number of days required for germination, N is the total number of germinated seeds. Time to 50% germination (T_{50}) was calculated according to the following formula of Coolbear *et al*. (1984) modified by Farooq *et al*. (2005):

$$T_{50} = t_i + [(N / 2 - n_i)\ (t_i - t_j)] / (n_i - n_j)$$

where N is the final number of seeds germinating and n_i, n_j cumulative number of seeds germinated by adjacent counts at times t_i and t_j, respectively when $n_i < N / 2 < n_j$.

At the end of the seeds germination tests, non-germinated seeds were tested for viability using 2,3,5-triphenyl tetrazolium chloride (TTC) solution, according to AOSA and SCST (2010). The seeds were soaked in 1% TTC solution for 24 hours in the dark at 25°C and a red stained embryo was used as an indication of seeds viability.

Biometric measurements. — After 30 days of seeds germination on Petri dishes soaked with NaF at concentrations of 9 mM, 19 mM and 29 mM and control, the length of hypocotyls and roots was measured in the seedlings of both species with the use of a ruler and a stereoscopic microscope (Leica M205 C) in the Leica Application Suite (3.8.0 build 878, LAS V3.8).

Determination of the free proline content of seedlings. — The free proline content of seedlings was determined by the ninhydrin spectrophotometric method described by Bates *et al*. (1973) with L-proline as the standard. Seedling samples were frozen in an ultrafreezer at -80°C and were then freeze-dried for 48 hours in the Alpha 1-2 LD laboratory freeze drier (Christ). The seedlings were pulverized in a mixer mill (MM200, Retsch). Proline was extracted by placing pulverized plant material in 3% (w/v) sulfosalicylic acid (n = 3). The samples were centrifuged at 14,000 rpm for 5 min (4°C). Free proline content was determined with the use of a supernatant. The reaction mixture contained 30 µl of the plant extract, 30 µl of the ninhydrin reagent and 30 µl of ice acetic acid. The mixture was immersed in a water bath with a temperature of 100°C for 1 h, and it was extracted with 60 µl of toluene. Free proline absorbance was read at 520 nm wavelength with the use of a UV/Vis spectrophotometer (Infinite M 200 Pro NanoQuant, Tecan), with toluene as the reference. Free proline content was determined based on standard curve values and expressed on a dry weight basis according to the following formula:

$$[(\mu g \text{ proline/ml x ml toluene})/115.5\ \mu g/\mu mole]/\ [(DW \text{ sample g})/5] = \\ = \mu moles \text{ proline/g DW}$$

Statistical analysis. — Parameters with normal distribution were analyzed by multifactorial analysis of variance to determine the influence of NaF on mean seed germination time and proline content. Homogeneous groups were identified by Tukey's test at a significance level of $p < 0.05$. Parameters without normal distribution were analyzed in the Kruskal-Wallis test to determine the effect of NaF on seeds germination capacity, the time to reach 50% germination (T_{50}) and seedling growth. A multiple comparison rank sum test was performed for all samples when significant differences were found between treatments ($p < 0.05$). The data were analyzed in the Statistica v. 12 program (StatSoft, Poland).

Results

Germination capacity of seeds. — At the tested concentrations, NaF did not reduce the germination capacity of *C. apetalus* seeds (Fig. 1). The above species was characterized by high seeds germination capacity (>97%) at all concentrations of NaF. The germination capacity of *C. quitensis* seeds was clearly influenced by the place of harvest (origin), and it was generally the lowest in greenhouse-grown seeds. Concentrations of 9 mM and 29 mM NaF significantly inhibited the seeds germination of *C. quitensis* harvested in greenhouse, 58% and 65%, respectively. The highest concentration of NaF (29 mM) significantly inhibited the seeds germination of *C. quitensis* harvested in Antarctica, and only 35% of the tested seeds germinated (Fig. 1).

Mean germination time (Pieper's index) and germination speed (T_{50}). — Sodium fluoride did not affect the mean germination time of *C. apetalus* seeds (Table 1). Seeds of *C. apetalus* germinated the fastest among the tested species (day 6,49). Sodium fluoride did not influence the mean germination time of greenhouse-grown *C. quitensis* seeds relative to control. However, *C. quitensis* seeds germinated faster under exposure to 9 mM (day 7.48) than 29 mM NaF (day 8.75). The seeds of *C. quitensis* from Antarctica germinated last (day 8.68). Exposure to 29 mM NaF significantly delayed the germination of *C. quitensis* seeds from Antarctica, which began only after 10 days of incubation (Table 1). Sodium fluoride had no significant effect on the time required to reach 50% germination of *C. apetalus* and *C. quitensis* seeds, except speed germination of *C. quitensis* seeds from Antarctica under exposure to 29 mM NaF (T_{50} was

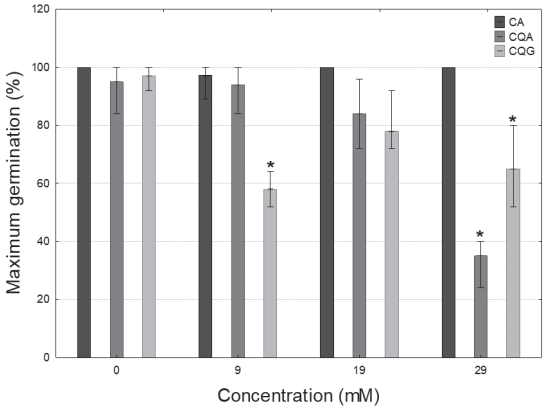

Fig. 1. The influence of NaF on the germination capacity of *C. apetalus* seeds harvested from greenhouse-grown plants (CA) and *C. quitensis* seeds harvested in Antarctica (CQA) and grown in a greenhouse (CQG). Mean values ± SD (n = 4). Asterisk indicates statistically significant differences at a significance level of $p < 0.05$.

Table 1

The influence of NaF on the mean germination time (Pieper's index) of *C. apetalus* seeds harvested from greenhouse-grown plants (CA) and *C. quitensis* seeds harvested in Antarctica (CQA) and grown in a greenhouse (CQG).

Concentration of NaF	Species		
	CA	CQA	CQG
0 mM	6.58^{EF}	8.68^{BC}	7.94^{CD}
9 mM	6.49^{F}	8.98^{B}	7.48^{DE}
19 mM	6.50^{F}	8.84^{BC}	8.11^{BCD}
29 mM	6.75^{EF}	10.09^{A}	8.75^{BC}

* Values marked with identical letters did not differ significantly at a significance level of $p < 0.05$ in Tukey's test ($n = 4$).

not calculated because of a too low germination percentage of seeds) (Table 2). The T_{50} for seeds of both species was quite fast. The time required to reach 50% germination of *C. apetalus* was 3.38–3.75 days. Seeds of *C. quitensis* from Antarctic had T_{50} of 3.79–4.25 days, while the T_{50} for seeds from greenhouse was 3.62–4.12 days (Table 2).

Seeds viability. — The tetrazolium viability test (TZ) revealed that the most non-germinated seeds of both species *Colobanthus* were alive (Table 3).

Seedlings growth. — Sodium fluoride concentrations of 19 mM and 29 mM significantly inhibited the growth of hypocotyls in *C. apetalus* seedlings (Figs 2A, 3c, d), and in these plants hypocotyls length was determined at 4.7 mm and

Table 2

The influence of NaF on the time (days) taken to achieve 50% germination (T_{50}) seeds of *C. apetalus* harvested from greenhouse-grown plants (CA) and seeds of *C. quitensis* harvested in Antarctica (CQA) and grown in a greenhouse (CQG).

Concentration of NaF	Species		
	CA	CQA	CQG
0 mM	3.75^{ns}	3.79^{ns}	3.79^{ns}
9 mM	3.50^{ns}	4.00^{ns}	3.62^{ns}
19 mM	3.38^{ns}	4.25^{ns}	3.75^{ns}
29 mM	3.75^{ns}	*	4.12^{ns}

^{ns} non-significant at a significance level of $p < 0.05$, * too low germination percentage of seeds (< 50%, see Figure 1).

Table 3

Percent of alive and dead non-germination seeds of *C. apetalus* and *C. quitensis* that had been incubated with TTC.

Species	Concentration of NaF	Alive %	Dead %
Seeds of *Colobanthus apetalus* harvested from a greenhouse	9 mM	100	0
Seeds of *Colobanthus quitensis* harvested in Antarctica	0 mM 9 mM 19 mM 29 mM	100 100 93.8 92.3	0 0 6.2 7.7
Seeds of *Colobanthus quitensis* harvested from a greenhouse	0 mM 9 mM 19 mM 29 mM	100 95.2 95.5 97.1	0 4.8 4.5 2.9

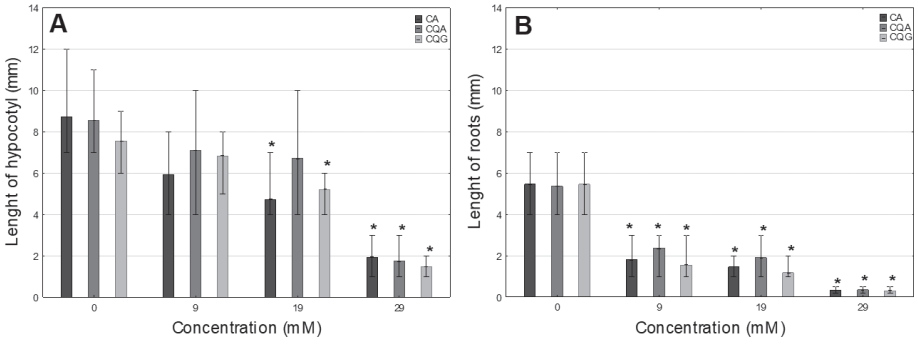

Fig. 2. The influence of NaF on the length of hypocotyls (**A**) and roots (**B**) in *C. apetalus* seedlings grown from a greenhouse seeds (CA) and in *C. quitensis* seedlings grown from seeds harvested in Antarctica (CQA) and greenhouse seeds (CQG). Mean values ± SD (n = 11). Asterisk indicates statistically significant differences at a significance level of $p < 0.05$.

1.9 mm, respectively. Concentrations of 19 mM and 29 mM NaF also significantly suppressed the growth of hypocotyls in *C. quitensis* seedlings grown from a greenhouse seeds (Figs 2A, 3m, n), and in these plants hypocotyls length was determined at 5.2 mm and 1.5 mm, respectively. In seedlings grown from *C. quitensis* seeds harvested in Antarctica, hypocotyls growth was significantly inhibited only under exposure to the highest concentration of NaF (29 mM). In these seedlings hypocotyls length reached 1.7 mm (Figs 2A, 3i).

All tested concentrations of NaF, 9 mM, 19 mM and 29 mM, significantly inhibited root growth in *Colobanthus* seedlings (Figs 2B, 3). Root length was determined at 0.34–1.80 mm in *C. apetalus* seedlings (Figs 2B, 3b–d),

Fig. 3. Effect of NaF on seedlings growth: *Colobanthus apetalus* from seeds harvested from greenhouse (**a–e**) and *Colobanthus quitensis* from seeds collected in Antarctica (**f–j**) and seeds from greenhouse (**k–o**) after 30 days of seeds germination on Petri dishes soaked with NaF at concentrations of 0 (control), 9 mM, 19 mM and 29 mM.

0.35–2.4 mm in *C. quitensis* seedlings grown from Antarctic seeds (Figs 2B, 3g–i), and 0.30–1.50 mm in *C. quitensis* seedlings grown from greenhouse seeds (Figs 2B, 3l–n).

Morphological symptoms. — The NaF treatment disrupted root growth and caused necrotic changes in plant tissues (Figs 3, 4). Seedlings of *C. apetalus* and *C. quitensis* which grown at concentration of 9 mM and 19 mM NaF had irregular and twisted shape roots. Seedlings of both species produced also few short root hairs at 9 mM and 19 mM NaF (Fig. 3b, c, g, h, l, m).

The seeds of both *Colobanthus* species also germinated under exposure to the highest concentration of NaF, 29 mM. However, most seedlings produced only short roots, and their further growth was inhibited (Fig. 3e, j, o). Seedlings which grown at concentrations of 29 mM NaF and produced hypocotyls and

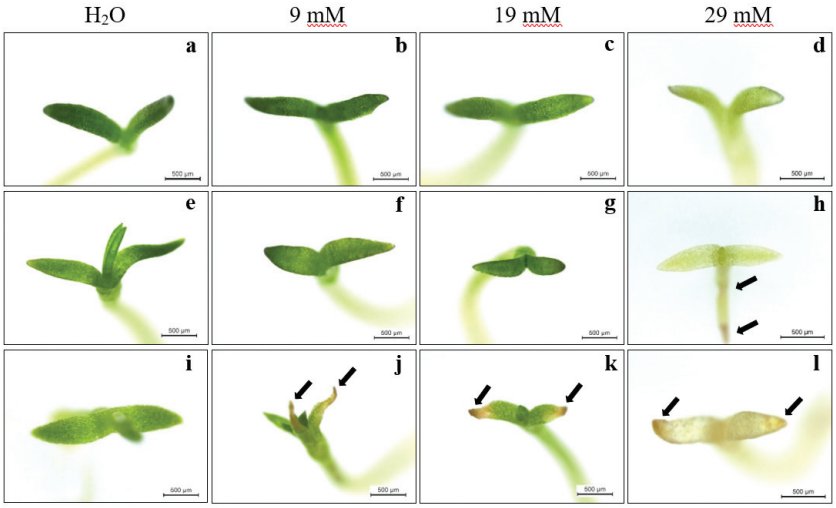

Fig. 4. Effect of NaF on the morphophysiology of *Colobanthus apetalus* seedlings from a greenhouse seeds (**a–d**), *Colobanthus quitensis* from seeds collected in Antarctica (**e–h**) and seeds from greenhouse cultivars (**i–l**) after 30 days of seeds germination on Petri dishes soaked with NaF at concentrations of 0 (control), 9 mM, 19 mM and 29 mM.

cotyledons had very short roots without root hairs (Fig. 3d, i, n). Concentration of 29 mM NaF also contributed to the growth of pathogenic fungi and necrosis of *Colobanthus* seedlings.

All tested concentrations of NaF, 9 mM, 19 mM and 29 mM, induced chlorosis and necrosis in the cotyledons of *C. quitensis* seedlings grown from greenhouse seeds (Figs 3l–n, 4j–l, arrows). In *C. quitensis* seedlings grown from Antarctic seeds, only the highest concentration of NaF induced chlorosis in cotyledons, and shoot and root necrosis (Fig. 4h, arrows). In *C. apetalus* seedlings grown from greenhouse seeds, cotyledons were clearly affected by chlorosis under exposure to the concentration of 29 mM NaF (Fig. 4d).

Proline content. — At all tested concentrations of NaF, proline content increased significantly in both *C. apetalus* and *C. quitensis* seedlings (Fig. 5). Proline content was the highest at 3.4 µmol·g^{-1}DW in the tissues of *C. apetalus* seedlings exposed to the concentration of 9 mM NaF. The content of free proline in *C. quitensis* seedlings grown from Antarctic seeds and greenhouse seeds increased with a rise in NaF concentration. The highest proline content was noted under exposure to NaF concentration of 19 mM, and it was determined at 3.2 µmol·g^{-1}DW in seedlings grown from Antarctic seeds and at 1.7 µmol·g^{-1}DW in seedlings grown from a greenhouse seeds (Fig. 5).

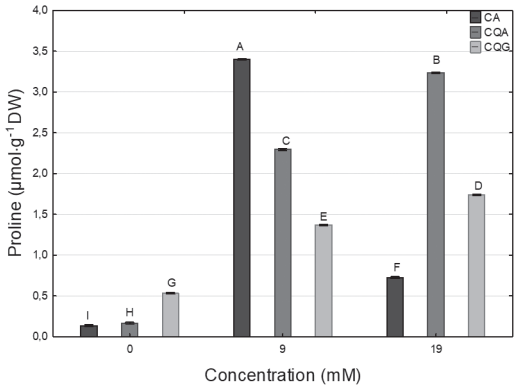

Fig. 5. The influence of NaF on the free proline content of *C. apetalus* seedlings grown from greenhouse seeds (CA) and *C. quitensis* seedlings grown from seeds harvested in Antarctica (CQA) and greenhouse-grown seeds (CQG). Proline content is expressed on a dry matter basis. Mean values ± SD (n = 3). Values marked with different letters differed significantly at a significance level of $p < 0.05$ in Tukey's test.

Discussion

The influence of NaF on the germination capacity of *C. apetalus* and *C. quitensis* seeds. — In flowering plants seed germination is influenced by numerous environmental factors. Stress tolerance is one of the key determinants of seed germination and plant growth. Fluorine and sodium fluoride inhibit seed germination in many unrelated plant species, both wild-growing and cultivated. The above correlation was demonstrated by Kaur and Duffus (1989) in three cereal species and by Gadi *et al*. (2012) in mung beans (*Vigna radiata*). Fluorine and sodium fluoride also inhibited germination in chickpeas (*Cicer arietinum*) (Datta *et al*. 2012; Sreedevi and Damodharam 2013) as well as shrub and small tree species, *Prosopis juliflora* (Saini *et al*. 2013) and *Cyamopsis teragonoloba* (Sabal *et al*. 2006).

In the group of the analyzed species of the genus *Colobanthus*, *C. apetalus* seeds were characterized by the highest germination capacity under exposure to NaF. The germination of *C. apetalus* seeds was not inhibited by any of the tested NaF concentrations (>97%). However, NaF inhibited germination of *C. quitensis* seeds. Seeds of *C. quitensis* collected from a greenhouse, were generally the most sensitive to this stressor. Only 58% and 65% of greenhouse-grown *C. quitensis* seeds germinated under exposure to 9 mM and 29 mM NaF, respectively. The germination of *C. quitensis* seeds from Antarctica was clearly compromised only at the highest concentration of NaF, 29 mM (35%). The tetrazolium viability test indicated that a high percentage of non-germinated seeds were viable. Probably NaF induced dormancy of seeds.

In our experiment, NaF partially inhibited the germination of *C. quitensis* seeds, but the germination capacity of both *C. quitensis* and *C. apetalus* seeds exposed to NaF remained high. The applied concentrations of NaF were relatively high compared to the concentrations of NaF used in the other studies, but despite the above, NaF did not completely suppress the germination of *C. quitensis* seeds. For example, Sreedevi and Damodharam (2013) reported that only 27% of *Cicer arietinum* seeds germinated under exposure to a much lower concentration of NaF, 75 ppm (1.8 mM), whereas the 100 ppm (2.4 mM) concentration of NaF completely inhibited seed germination in the analyzed species. Moreover, Gadi *et al*. (2012) demonstrated that, 82% of *Vigna radiata* seeds germinated under exposure to NaF at a concentration of 1 mM (the highest concentration used in the cited study). Sabal *et al*. (2006) reported that only 20% of *Cyamopsis teragonoloba* seeds germinated under the influence of 25 µM NaF, whereas exposure to 30 µM NaF completely inhibited germination in the above species. Our results indicate that seeds of *C. apetalus* and *C. quitensis* are highly resistant to NaF.

The influence of NaF on mean germination time (Pieper's index) and germination speed (T_{50}). — In this study, NaF did not influence on the time required to reach 50% germination of seeds, except seeds of *C. quitensis* from Antarctica under exposure to 29 mM NaF (only 35% of seeds were germinated and T_{50} was not calculated). The speed of germination represented by T_{50} was similar for both *Colobanthus* species and was quite fast. Sodium fluoride also did not influence on the mean germination time (Pieper's index) of *C. apetalus* seeds (6.49–6.75 days) and *C. quitensis* seeds grown in a greenhouse relative to control (7.94–8.75 days). However, greenhouse-grown *C. quitensis* seeds germinated faster under exposure to NaF concentration of 9 mM (7.48 days) than 29 mM (8.75 days). Sodium fluoride at a concentration of 29 mM significantly prolonged the germination of *C. quitensis* seeds from Antarctica (10.09 days). Similar effects have been observed in *Cicer arietinum* (Datta *et al*. 2012). The germination time of *Cicer arietinum* seeds was prolonged with an increase in F concentration.

It should also be noted that the germination of *C. quitensis* seeds from Antarctica was significantly inhibited only at NaF concentration of 29 mM (only 35% of seeds were germinated), which could be attributed to the adaptive strategy of the species. The seeds of *C. quitensis* do not have physiological features to enable wind-aided dispersal, therefore, they are not able to fly long distances, and they are dispersed gradually (Venable and Lawlor 1980). Delayed germination is probably an adaptive mechanism which enables plants growing in harsh environments to wait for more supporting conditions and which prevents all diaspores from germinating simultaneously. The above could increase the reproductive success of *C. quitensis* in the severe and unpredictable climate of Antarctica.

The influence of NaF on seedling growth. — Research has demonstrated that F and its derivatives inhibit cell elongation and plant growth. Fluorine and sodium fluoride suppressed stem and root growth in *Cicer arietinum* (Datta *et al*. 2012), *Prosopis juliflora* (Saini *et al*. 2013), *Hordeum vulgare, Triticum aestivum* and *Oryza sativa* (Kaur and Duffus 1989) as well as in *Vigna radiate* (Gadi *et al*. 2012). In the present study, NaF inhibited the growth of seedlings of polar and subpolar plants of the genus *Colobanthus*. At concentrations of 19 mM and 29 mM NaF significantly limited the hypocotyl growth of *C. apetalus* and *C. quitensis* seedlings grown from greenhouse seeds. Only the highest concentration of NaF (29 mM), significantly limited the hypocotyl growth of *C. quitensis* seedlings grown from Antarctic seeds. In our experiment root system of *C. apetalus* and *C. quitensis* was more sensitive to the stress caused by NaF. All concentrations of NaF (9 mM, 19 mM, 29 mM), significantly suppressed root growth in both *Colobanthus* species. Similar results were observed in *C. quitensis* seedlings response to copper (Cuba-Díaz *et al*. 2017a). Our results show, that seedlings of *C. quitensis* from Antarctic seeds characterized by the most favorable growth and development. Seedlings have the generally longest hypocotyls and roots at NaF concentrations. While *C. quitensis* seedlings grown from greenhouse seeds were the most sensitive to NaF. In our study, all of the tested NaF concentrations led to chlorosis/necrosis of cotyledon tips in *C. quitensis* seedlings grown from greenhouse seeds. We also observed chlorosis in cotyledons of *C. apetalus* seedlings grown from greenhouse seeds and *C. quitensis* seedlings grown from Antarctic seeds under exposure only to the highest concentration of NaF (29 mM). The accumulation of fluorine in cotyledons probably disrupted metabolic processes. Sodium fluoride also caused significant damage to cotyledons in *Hypericum perforatum* (Fornasiero 2001; 2003), *Populus tremuloides* (Kamaluddin and Zwiazek 2003) and *Salicornia brachiata* (Reddy and Kaur 2008), including necrosis and changes in the color of assimilative parenchyma from green to red. Cuba-Díaz *et al*. (2017b) have recently shown also that concentration of 100 mM and 200 mM NaCl induced chlorosis at the leaf base and in leaf tips of *C. quitensis*.

Variation in the germination capacity of seeds, germination rate and seedlings growth between seeds of *C. quitensis* collected from Antarctica and greenhouse, may be caused by differences in seed morphology. The size, shape and seed mass are important determinant of the relative success in later phases of the life cycle of plants (Tripathi and Khan 1990). The seeds of *C. quitensis* are characterized by somatic polymorphism. Seed polymorphism can increase plant resistance to environmental stressors (Silvertown 1984). The seeds of *C. quitensis* harvested in Antarctica (Kellman-Sopyła *et al*. 2017) have significantly higher 1000-seed-weight, length and width than greenhouse-grown seeds (Kellmann-Sopyła *et al*. 2015). Smaller seeds have thinner seed coat for the embryos to penetrate and may germinate more quickly. Whereas, heavy seeds have larger

reserve that may contribute to better growth and survival of seedlings (Dolan 1984). Seedlings size is related with food reserves and energy content of seeds in other species such as *Quercus dealbata* and *Q. griffit* (Tripathi and Khan 1990), *Virola surinamensis* (Howe and Richter 1982) and *Pinus taeda L.* (Dunlop and Barnett 1983). However, there are no major differences in seeds mass of *C. apetalus* (53 mg) (Dulska *et al.* data not published) and *C. quitensis* seeds collected from Antarctica (51–53 mg). Differences in the germination capacity of seeds and seedlings growth between *Colobanthus* species may be caused by differences in accumulation of protein, lipid or carbohydrate, but currently there is no information about the anatomy and physiology of *C. apetalus*. Flint and Palmblad (1978) reported that, an increased proportion of protein and carbohydrate provides the readily available energy which stimulates germination seeds of *Heterotheca grandiflora*.

The influence of NaF on the proline content of *Colobanthus* seedlings. — Proline accumulation is an adaptive strategy of plants to stressful environment. For example, proline accumulation maintains the osmotic balance, scavenges excess free radicals and stabilizes cell membrane structure and function (Khan *et al.* 2015). Schat *et al.* (1997) who analyzed proline content in response to heavy metal in nontolerant and metal-tolerant ecotypes of *Silene vulgaris* (Caryophyllaceae) reported that proline accumulation was higher in the metal-tolerant ecotypes. Proline also does seem to play an important role in the mechanism of NaF tolerance in *C. apetalus* and *C. quitensis*. In our study, the content of free proline in seedlings of both *Colobanthus* species increased significantly under exposure to NaF. Proline concentration was the highest in the tissues of *C. apetalus* seedlings exposed to 9 mM NaF. Proline accumulation in the seedlings of *C. quitensis* grown from Antarctic and greenhouse seeds, increased with a rise in NaF concentration. Our findings are similar to accumulation of free proline under exposure to NaF observed in *Vigna radiate* by Gadi *et al.* (2012). The accumulation of proline in response to NaF stress has been also described in *Azolla microphylla* and *A. filiculoides* (Eyini *et al.* 1999) as well as in *Cicer arietinum* (Datta *et al.* 2012). Cuba-Díaz *et al.* (2017a) reported that proline was also accumulated in the leaves and stems of *C. quitensis* exposed to high concentrations of $CuSO_4$. Furthermore, the second flowering plant native to Antarctica, *Deschampsia antarctica*, accumulated proline under the influence of salt stress (Tapia-Valdebenito *et al.* 2016).

Conclusions

Our results demonstrated a high germination capacity of both *Colobanthus* species seeds. Sodium fluoride partially inhibited the germination of *C. quitensis* seeds, but to our knowledge this is the first report on NaF tolerance using so high concentrations. The applied concentrations of NaF did not completely suppress the germination of *C. quitensis* seeds. This study revealed that NaF mainly inhibited the root growth. According that, root system is more sensitive to the stress caused by NaF of both *Colobanthus* species. Observed chlorosis in cotyledons and increase proline content may be stress indicators. We suggest that despite certain symptoms of stress, both species are highly resistant to NaF.

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