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ORIGINAL RESEARCH PAPER

The effects of methanesulfonic acid on seed germination and morphophysiological changes in the seedlings of two *Colobanthus* species

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Abstract
The effect of methanesulfonic acid (MSA) on the morphophysiology and biochemistry of the subantarctic species *Colobanthus apetalus* and the Antarctic species *Colobanthus quitensis* was examined. We evaluated the effects of various concentrations of MSA on the germination capacity and germination rate of seeds, seedling growth, chlorophyll fluorescence in cotyledons, and the proline content of seedlings under laboratory conditions at temperatures of 20°C (day) and 10°C (night) with a 12/12 h photoperiod. The examined *C. apetalus* seeds were grown in a greenhouse, and *C. quitensis* seeds were harvested in Antarctica and grown in a greenhouse (Olsztyn, Poland). The seeds of *C. apetalus* were characterized by the highest germination capacity and the highest germination rate, whereas *C. quitensis* seedlings were characterized by the most favorable growth and development. Only the highest concentrations of MSA decreased the intensity of chlorophyll fluorescence in the cotyledons of both *Colobanthus* species. The proline content of *C. apetalus* and *C. quitensis* seedlings increased significantly after MSA treatments. The results of this study clearly indicated that *Colobanthus quitensis* is more resistant to chemical stress induced by MSA. This is a first study to investigate the influence of MSA on the morphophysiology and biochemistry of higher plants.

Keywords
Colobanthus apetalus; *Colobanthus quitensis*; sub-Antarctic; Antarctica; methanesulfonic acid; environmental stress

Introduction

Terrestrial Antarctic ecosystem development is limited to only small ice-free areas, mainly concentrated on the coastal zones of the maritime Antarctic. Low temperatures, limited liquid water access, desiccating winds, high levels of ultraviolet-B radiation, poorly developed soils [1,2], high salinity in many locations, and many other local stressors significantly influence terrestrial communities. Thus, characteristic features of the Antarctic include poverty of flowering plants, whose number is limited to only

two native species: *Colobanthus quitensis* (Kunth) Bartl. and *Deschampsia antarctica* Desv. (Poaceae), and one alien taxon *Poa annua* L. (Poaceae) [3–7]. The subpolar zone is characterized by milder environmental conditions and more diverse plant life. One of the species found in this region is *Colobanthus apetalus* (Labill.) Druce [8,9].

Tolerance to environmental stressors determines seed germination and the growth and development of plants. Tolerance to abiotic stress is conditioned by various factors, including biochemical factors (proline, soluble sugars), physiological factors (photosynthesis), plant growth rate (height), and biomass production [10].

Polar plants are model species for investigating responses to environmental stressors because they are continuously exposed to harsh and changeable microhabitat conditions, and their adaptive capabilities have been studied for many years [2,5,11,12]. According to the literature, *C. quitensis* has developed unique anatomical and morphophysiological mechanisms that effectively minimize the adverse consequences of stress and enable the species to survive in the difficult climate of Antarctica [13–19]. However, stress tolerance of the related subpolar *C. apetalus* has not been investigated to date.

The growth and development of Antarctic plants can also be inhibited by chemical stress resulting from exposure to, for example, methanesulfonic acid (CH_3SO_3H; MSA).

The origin of MSA in Antarctica

Globally, the concentration of MSA in air is low, but MSA is found in significant concentrations in gas and aerosol phases over oceans and in coastal regions [3]. In 2001, Rankin and Wolff [20] reported MSA levels of 37.5 ng m^{-3} in summer and 0.9 ng m^{-3} in winter at the Halley Antarctic station situated on the Weddell Sea coast. Because of its extreme solubility, MSA is effectively scavenged by water droplets in the atmosphere [21], and falls to the Earth's surface with rain and snow.

MSA plays an important role in the global sulfur cycle, and it is characteristic of marine biogenic activity. MSA is the product of photochemical oxidation of dimethyl sulfide (CH_3SCH_3; DMS) in the atmosphere. Dimethyl sulfide is produced through the decomposition of dimethylsulfoniopropionate [$(CH_3)_2S+CH_2CH_2COO^-$; DMSP), an algal osmolyte [22]. The annual global estimates of MSA from DMS vary between 20 and 50 Tg [23].

Methanesulfonic acid (boiling point 167°C at 10 mm Hg) is a strong ($pK_a = -1.9$) and stable acid [24]. According to some authors, hydrated clusters of MSA in coastal areas can contribute to the formation of new particles [25,26]. There are no other known sources of MSA in marine air; therefore, MSA in ice cores could be regarded as an indicator of past oceanic emissions of DMS [27].

MSA and living organisms

Some bacterial strains are capable of utilizing MSA, a C_1-compound, as their only source of carbon, sulfur, and energy [28–32]. According to Biedlingmaier and Schmidt [33], the green algae *Chlorella fusca* is also able to metabolize MSA and other sulfonic acids.

However, the interactions between MSA and angiosperms have never been researched. Therefore, the goal of this study was to examine the influence of MSA on the morphophysiology and biochemistry of *C. apetalus* and *C. quitensis*. We hypothesized that *C. apetalus* and *C. quitensis* are highly resistant to MSA stress and develop mechanisms of tolerance to this stressor. To test this hypothesis, we used a high concentration of MSA and examined its influence on the germination capacity and germination rate of *C. apetalus* and *C. quitensis* seeds, seedling growth, chlorophyll fluorescence in cotyledons, and the proline content of plant tissues.

Material and methods

Plant material

Seeds of *Colobanthus apetalus* were harvested on the southeastern shore of Lago Roca in the Tierra del Fuego National Park, in the vicinity of Ushuaia (68°30′ S, 54°50′ W, Argentina). Seeds of *Colobanthus quitensis* were harvested in Lion's Rump, King George Bay (62°08′ S, 58°08′ W; King George Island, Western Antarctic). The collected seeds were sown in a greenhouse at the University of Warmia and Mazury in Olsztyn, Poland. Greenhouse plants were grown at a temperature of 20°C with a 16/8 h photoperiod, in pots filled with a 1:1:1 mixture of hortisol, sand, and peat. The experiment was performed on *C. apetalus* seeds collected from greenhouse-grown plants in the summer of 2015 (June-September), as well as *C. quitensis* seeds harvested on King George Island in March 2012 and from greenhouse-grown plants in the summer of 2015 before the main experiment on seed viability was tested.

Experimental conditions and MSA treatments

The seeds of *C. apetalu*s and *C. quitensis* were germinated for 30 days on Petri dishes lined with filter paper soaked with deionized water as the control and with 5 mL of an aqueous solution of MSA at concentrations of 2 mM, 4 mM, and 6 mM. Every tested variant consisted of 36 *C. apetalus* seeds (nine seeds per plate because of a limited number of seeds) and 100 *C. quitensis* seeds (25 seeds per plate). The tested concentrations were determined empirically during the preliminary experiment. A total of 144 *C. apetalus* seeds and 400 *C. quitensis* seeds were used in germination tests. The seeds were germinated at a temperature of 20/10°C with a 12/12 h photoperiod, respectively. After 30 days of the experiment, the percentage (%) of germinated seeds was calculated as follows: $G\,(\%) = (A/B) \times 100$, where: A – total number of germinated seeds, B – total number of tested seeds.

Mean germination time (Pieper's index) was calculated according to the formula below: $\textit{Pieper's index} = \sum(nt)/N$, where: n – number of germinated seeds on a given day, t – number of days required for germination, N – total number of germinated seeds.

After germination, the viability of nongerminated seeds was determined using a tetrazolium test according to AOSA and SCST [34].

Biometric measurements

The length of hypocotyls and radicles of *C. apetalus* and *C. quitensis* plants grown on the MSA medium for 30 days was measured with a ruler and under a stereoscopic microscope (Leica M205 C) in the Leica Application Suite (3.8.0 build 878, LAS V3.8).

Fluorescence measurements

Chlorophyll fluorescence in cotyledons was measured in vivo under a confocal laser scanning microscope (Leica TCS SP5). Before measurements, seedlings were adapted to dark for 30 minutes in a dark room. Chlorophyll fluorescence was excited with an argon laser (488 nm), and was measured at a wavelength of 650–695 nm (red spectral region; Fr) and 700–795 nm (far-red spectral region; FFr). The intensity of chlorophyll fluorescence was measured in each treatment. All measurements were performed on three cotyledons from three different seedlings ($n = 3$), at three points in every cotyledon: base, middle, and tip. The chlorophyll fluorescence intensity ratio (FFr/Fr) was calculated for each point in every cotyledon and mean values were determined. Changes in the mean intensity of chlorophyll fluorescence were measured with the Stack Prolife tool (Leica Application Suite Advanced Fluorescence, 2.4.1 build 6384).

Proline content of seedlings

Proline content was determined spectrophotometrically according to the ninhydrin method described by Bates et al. [35], using L-proline as the standard. Before the assay, seedlings were placed in an ultrafreezer at −80°C and were freeze-dried for 48 h in the Alpha 1-2 LD laboratory freeze drier (Martin Christ Gefriertrocknungsanlagen GmbH, Germany). The seedlings were then pulverized into fine powder using a mixer mill (MM 200, Retsch, Germany). Proline was extracted by placing pulverized tissue in 3% (w/v) sulfosalicylic acid ($n = 3$). The samples were centrifuged at 14,000 rpm for 5 min (4°C), and 30 µL of the supernatant was added to 30 µL of the ninhydrin reagent and 30 µL of ice acetic acid. The reaction mixture was incubated for 1 h (100°C) and 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, Switzerland), with toluene as the reference.

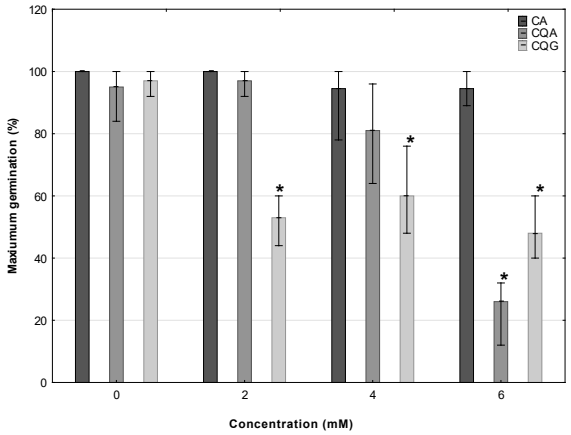

Fig. 1 The influence of MSA on the germination capacity of *Colobanthus apetalus* seeds collected from greenhouse-grown plants (CA) and *Colobanthus quitensis* seeds collected in Antarctica (CQA) and from greenhouse-grown plants (CQG). Mean values ±*SD* ($n = 4$).

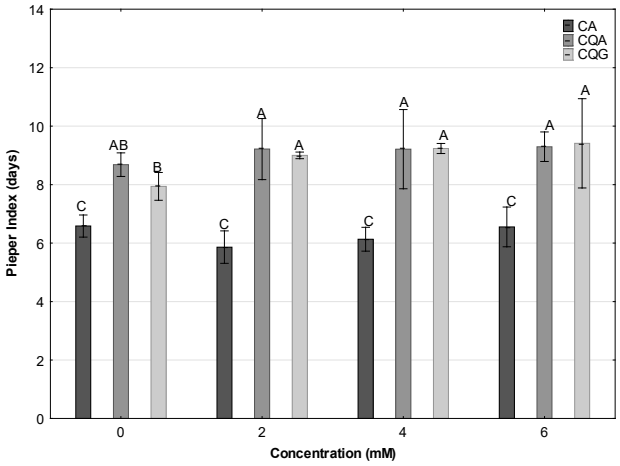

Fig. 2 The influence of MSA on the mean germination time (Pieper's index) of *Colobanthus apetalus* seeds collected from greenhouse-grown plants (CA) and *Colobanthus quitensis* seeds collected in Antarctica (CQA) and from greenhouse-grown plants (CQG). Mean values ±*SD* ($n = 4$). Values marked with identical letters did not differ significantly at $p < 0.05$ in Tukey's test.

Statistical analysis

The data were analyzed statistically using Statistica v.12 software (StatSoft, Poland). A one-way analysis of variance (ANOVA) was conducted to evaluate the influence of MSA on mean seed germination time, chlorophyll fluorescence, and proline content. Homogeneous groups were identified by Tukey's test at a significance level of $p < 0.05$. The influence of MSA on seed germination capacity and seedling growth was determined using the Kruskal–Wallis test. A rank sum test for multiple comparisons was performed for all samples when significant differences were found among treatments ($p < 0.05$).

Results

Germination test

The seeds of *C. apetalus* were characterized by the highest germination capacity, and they germinated most rapidly (Fig. 1, Fig. 2). At the tested concentrations, MSA did not reduce the germination capacity of *C. apetalus* seeds, and the percentage of germinated seeds exceeded 94.5%. The germination capacity of *C. quitensis* seeds was significantly influenced by the place of harvest. The seeds of *C. quitensis* collected in a greenhouse were characterized by the lowest germination capacity. All tested concentrations of MSA (2 mM, 4 mM, 6 mM) significantly inhibited the germination of greenhouse-grown *C. quitensis* seeds by 53%, 60%, and 48%, respectively. The highest concentration of MSA (6 mM) significantly inhibited the germination of *C. quitensis* seeds harvested in Antarctica, and only 26% of the seeds germinated (Fig. 1). Methanesulfonic acid did not affect the mean germination time of *C. apetalus* seeds collected in the greenhouse (6 days) and *C. quitensis* seeds harvested in Antarctica (9 days) (Fig. 2). However, MSA treatments significantly delayed the germination of greenhouse-grown *C. quitensis* seeds. At all tested concentrations of MSA, greenhouse-grown *C. quitensis* seeds began to germinate only after 9 days of incubation (Fig. 2). The tetrazolium viability test revealed that most nongerminated seeds of both tested species were alive (Tab. 1).

Tab. 1 The percentage of viable and nonviable nongerminated seeds of *Colobanthus apetalus* and *Colobanthus quitensis* after incubation with TTC

Species	Concentration of MSA	Viable %	Nonviable %
Colobanthus apetalus seeds collected from a greenhouse	4 mM	100	0
	6 mM	100	0
Colobanthus quitensis seeds collected in Antarctica	H_2O	100	0
	2 mM	100	0
	4 mM	95	5
	6 mM	93	7
Colobanthus quitensis seeds collected from a greenhouse	H_2O	100	0
	2 mM	94	6
	4 mM	97	3
	6 mM	92	8

Seedlings growth

MSA concentrations of 4 mM and 6 mM significantly inhibited the growth of hypocotyls in *C. apetalus* seedlings grown from greenhouse seeds and *C. quitensis* seedlings grown from Antarctic seeds (Fig. 3A, Fig. 4C,D,H,I). In the above treatments, hypocotyl length was determined to be 4.45 mm and 2.45 mm in *C. apetalus* seedlings for MSA concentrations of 4 mM and 6 mM, respectively, and 5.0 mm and 2.45 mm in *C. quitensis* seedlings grown from Antarctic seeds, respectively. Only the highest concentration of MSA (6 mM) significantly inhibited hypocotyl growth in *C. quitensis* seedlings grown from greenhouse seeds. In these seedlings, hypocotyl length was determined to be 3.45 mm (Fig. 3A, Fig. 4N).

All tested concentrations of MSA significantly inhibited root growth in both *Colobanthus* species seedlings (Fig. 3B, Fig. 4). Root length was determined to be 0.43–2.09 mm in *C. apetalus* seedlings (Fig. 3B, Fig. 4B–D), 0.41–2.09 mm in *C. quitensis* seedlings grown from Antarctic seeds (Fig. 3B, Fig. 4G–I), and 0.55–2.63 mm in *C. quitensis* seedlings grown from greenhouse seeds (Fig. 3B, Fig. 4L–N).

At concentrations of 2 mM and 4 mM, MSA disrupted root growth in both *C. apetalus* and *C. quitensis* seedlings (Fig. 4B,C,G,H,L,M). Their radicles were deformed, had a twisted shape, and produced several short root hairs. The seeds of *C. apetalus* and *C. quitensis* also germinated under exposure to the highest concentration of MSA. However, most seedlings grown under exposure to 6 mM MSA produced only short radicles, and

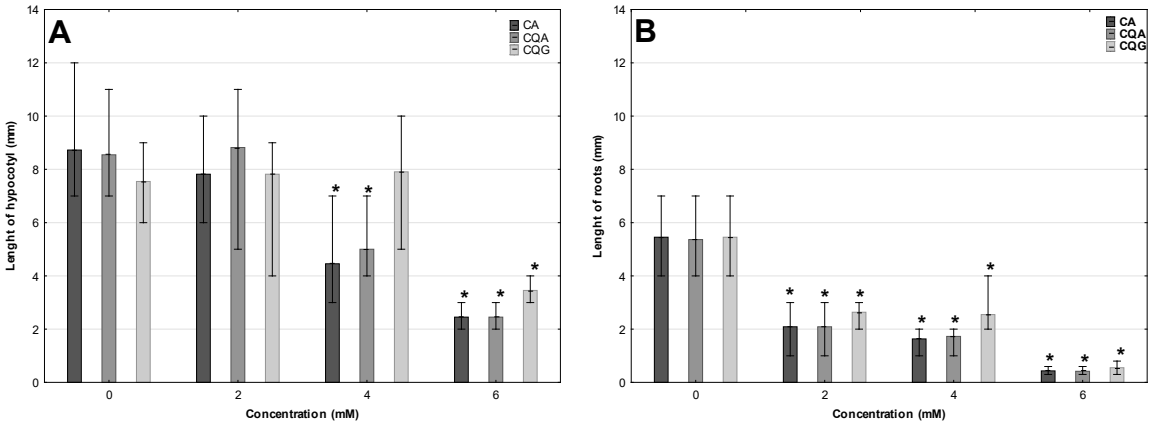

Fig. 3 The influence of MSA on the length of hypocotyls (**A**) and radicles (**B**) in *Colobanthus apetalus* seedlings grown from greenhouse seeds (CA) and in *Colobanthus quitensis* seedlings grown from seeds collected in Antarctica (CQA) and from greenhouse seeds (CQG). Mean values ±*SD* (n = 11). Asterisks indicate statistically significant differences at $p < 0.05$ in Tukey's test, one-way ANOVA.

Fig. 4 The effect of MSA (H_2O, 2 mM, 4 mM, 6 mM) on the growth of *Colobanthus apetalus* seedlings grown from greenhouse seeds (**A–E**) and *Colobanthus quitensis* seedlings grown from Antarctic seeds (**F–J**) and greenhouse seeds (**K–O**) after 30 days of incubation on Petri dishes.

their additional growth was inhibited (Fig. 4E,J,O). Seedlings that produced hypocotyls and cotyledons also developed very short radicles without root hairs (Fig. 4D,I,N).

Intensity of chlorophyll fluorescence

The highest concentration of MSA (6 mM) induced chlorosis and necrosis in the cotyledons of both *Colobanthus* species (Fig. 5D,H,L). The above treatment significantly decreased the intensity of chlorophyll fluorescence in the cotyledons of *C. apetalus* and *C. quitensis* (Fig. 6). The greatest decrease in chlorophyll fluorescence was noted in the cotyledons of *C. apetalus* seedlings grown from greenhouse seeds, where the FFr/Fr ratio increased markedly to 0.33. Under exposure to the highest concentration of MSA, the FFr/Fr ratio increased to 0.23 in *C. quitensis* seedlings grown from Antarctic seeds and to 0.24 in *C. quitensis* seedlings grown from greenhouse seeds (Fig. 5D,H,L, Fig. 6).

Proline content

Proline content increased significantly in both *Colobanthus* seedlings at all tested concentrations of MSA (Fig. 7). Proline content was highest in the tissues of *C. apetalus* seedlings under exposure to 2 mM MSA, and it was determined to be 4.15 μmol g^{-1} DW. Proline accumulation in the seedlings of *C. quitensis* grown from Antarctic and greenhouse seeds increased with a rise in MSA concentration. The highest proline content of *C. quitensis* seedlings grown from Antarctic seeds and greenhouse seeds was observed under exposure to 4 mM MSA, and it was determined to be 3.27 μmol g^{-1} DW and 3.31 μmol g^{-1} DW, respectively (Fig. 7).

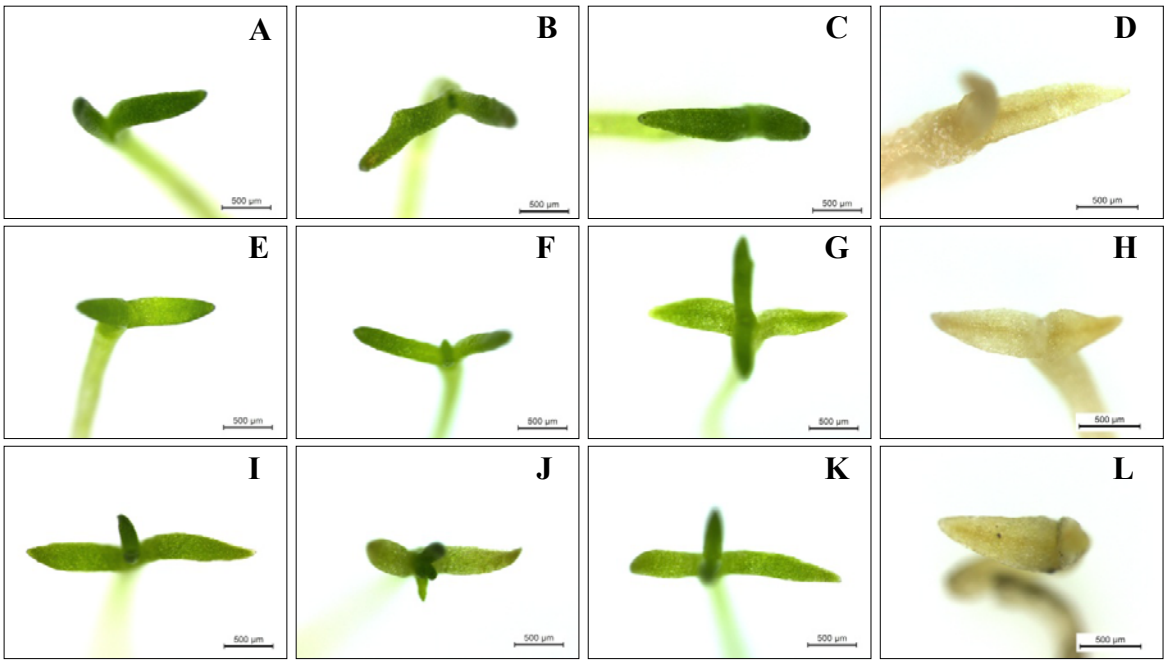

Fig. 5 The effect of MSA (H_2O, 2 mM, 4 mM, 6 mM) on the morphophysiology of *Colobanthus apetalus* seedlings grown from greenhouse seeds (**A–D**), *Colobanthus quitensis* seedlings grown from Antarctic seeds (**E–H**), and greenhouse seeds (**I–L**) after 30 days of incubation on Petri dishes.

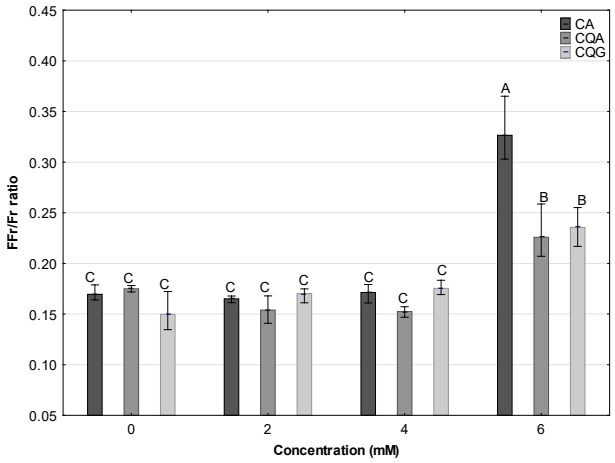

Fig. 6 The influence of MSA on chlorophyll fluorescence in the cotyledons of *Colobanthus apetalus* seedlings grown from greenhouse seeds (CA) and *Colobanthus quitensis* seedlings grown from Antarctic seeds (CQA) and greenhouse seeds (CQG). Mean values ±*SD* ($n = 3$). Values marked with identical letters did not differ significantly at $p < 0.05$ in Tukey's test, one-way ANOVA.

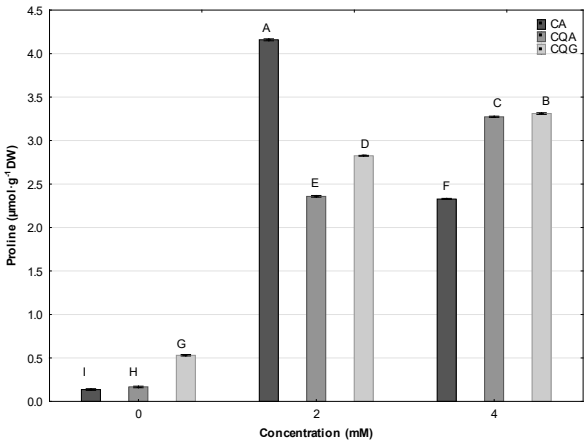

Fig. 7 The influence of MSA on the proline content of *Colobanthus apetalus* seedlings grown from greenhouse seeds (CA) and *Colobanthus quitensis* seedlings grown from Antarctic seeds (CQA) and greenhouse seeds (CQG). Proline content is expressed on a dry matter basis. Mean values ±*SD* ($n = 3$). Values marked with different letters differ significantly at $p < 0.05$ in Tukey's test.

Discussion

Germination capacity and mean germination time of *C. apetalus* and *C. quitensis* seeds exposed to MSA

In the present study, none of the tested concentrations of MSA decreased the germination capacity and did not affect the mean germination time of *C. apetalus* seeds. Our results indicate that germination capacity and germination rate were higher in *C. apetalus* seeds than in *C. quitensis* seeds under exposure to MSA. The highest concentration of MSA significantly inhibited the germination of *C. quitensis* seeds collected in Antarctica. However, MSA did not affect the mean germination time of this species. The greenhouse-grown seeds of *C. quitensis* collected were most sensitive to MSA stress. All tested concentrations of MSA significantly inhibited the germination of *C. quitensis* seeds. Nazarenko et al. [36] demonstrated that DMS significantly decreased the germination capacity of *Triticum aestivum* L. seeds. Additionally, the germination of *Rhus coriaria* seeds was significantly reduced after being soaked in sulfuric acid because of loss of seed viability [37]. In our study, the tetrazolium viability test revealed that a high percentage of nongerminated seeds were viable; thus, methanesulfonic acid induced dormancy in *C. quitensis* seeds. It should also be noted that MSA treatments significantly delayed the germination of greenhouse-grown *C. quitensis* seeds, which began to germinate only after 9 days of incubation at all tested concentrations of MSA. Delayed germination could be an adaptive mechanism in *C. quitensis*, which enables the species to survive in a hostile environment. The influence of environmental stressors such as salinity and drought on delayed seed germination in other species has been already documented by many authors (e.g., [38,39]). Our findings suggest that the seeds of *C. apetalus* and *C. quitensis* are characterized by high germination capacity under exposure to MSA. The analyzed acid inhibited, but did not completely suppress the germination of *C. quitensis* seeds, which suggests that *C. quitensis* seeds are also highly resistant to MSA stress.

The effect of MSA on the growth of *Colobanthus* seedlings

In our study, MSA concentrations of 4 mM and 6 mM significantly inhibited the growth of hypocotyls in *C. apetalus* in seedlings grown from greenhouse seeds and *C. quitensis* seedlings grown from Antarctic seeds. Only the highest concentration of MSA significantly inhibited the growth of hypocotyls in *C. quitensis* seedlings grown from greenhouse seeds. Interestingly, the inhibitory effect of MSA was observed mainly in root tissues. All concentrations of MSA significantly inhibited root growth in *C. apetalus* and *C. quitensis* seedlings. The analyzed acid not only inhibited root growth, but also caused visible damage to the morphological structure of radicles. The radicles of both *Colobanthus* species were deformed; they had a twisted shape and produced several short root hairs or were devoid of root hairs. Our results indicate that the root systems of both analyzed species were sensitive to MSA stress. These analyses were confirmed by observations of other authors observing inhibition of growth of seedlings in other species after exposition to SO_2 [40,41]. Moreover, Yi et al. [42] demonstrated that highly concentrated SO_2 hydrates (0.5–30.0 mM) inhibited the growth of *Hordeum vulgare* roots by delaying the cell cycle and inducing cell death. However, at a concentration of 0.1 mM, SO_2 hydrates exerted a minor stimulatory effect on the growth of *Hordeum vulgare* roots. A similar effect was observed by Gosh et al. [43] in whose study, *Passiflora edulis* var. *flavicarpa* seeds soaked in sulfuric acid produced the longest shoots, roots, and tallest seedlings.

According to López-Bucio et al. [44], plants are particularly sensitive to changes in nutrient availability during key developmental processes, such as root-hair formation, primary root growth, and lateral root formation. Plants may modify their root structure to maximize the utilization of the available nutrients and survive in inhospitable environments.

This study also demonstrated that the growth and development of *C. quitensis* seedlings was optimized in MSA treatments. Under exposure to MSA, *C. quitensis* seedlings generally produced longer hypocotyls and radicles than *C. apetalus* seedlings.

The effect of MSA on the intensity of chlorophyll fluorescence in the cotyledons of both *Colobanthus* species

Photosynthesis is particularly sensitive to environmental stressors that can alter or damage the photosynthetic apparatus [45]. Photosynthetic efficiency is determined by measuring chlorophyll fluorescence [46], which is a reliable indicator of plant health and the chlorophyll content of plant tissues. Chlorophyll fluorescence is also indicative of plant responses to stress [40,41,47,48].

In our experiment, MSA induced a dramatic decrease in the intensity of chlorophyll fluorescence in *C. apetalus* and *C. quitensis* seedlings. Under exposure to the highest concentration of MSA, the FFr/Fr ratio significantly decreased in both *Colobanthus* species. *Colobanthus apetalus* seedlings were characterized by the greatest drop in chlorophyll fluorescence under the influence of MSA. The FFr/Fr ratio in *C. apetalus* cotyledons increased to 0.33. However, the photosynthetic apparatus of *C. quitensis* seedlings was more resistant to MSA stress than that of *C. apetalus*. Under exposure to 6 mM of MSA, the FFr/Fr ratio increased to 0.23 in *C. quitensis* seedlings grown from Antarctic seeds and to 0.24 in *C. quitensis* seedlings grown from greenhouse seeds. MSA acid also led to chlorosis in the cotyledons of both *Colobanthus* seedlings. The accumulation of MSA in the cotyledons of *C. apetalus* and *C. quitensis* probably induced necrotic changes in assimilative parenchyma by damaging the photosynthetic apparatus. Similar results were reported by Liu et al. [48], in whose study, exposure to SO_2 caused leaf wilt, chlorosis, and leaf apex necrosis in *Triticum aestivum* L. Sulfur dioxide also caused chlorosis in the cotyledons and primary needles of *Pinus resinosa* Ait. [40] and in the leaves of *Acer saccharinum* L. [41].

The effect of MSA on the proline content of *C. apetalus* and *C. quitensis* seedlings

In the current study, proline accumulation was significantly affected by MSA stress. The content of free proline increased with a rise in MSA concentration in both species seedlings. Proline content was highest in the tissues of *C. apetalus* seedlings exposed to 2 mM of MSA. In *C. quitensis* seedlings, the highest proline content was noted at the MSA concentration of 4 mM. Our data suggest that proline accumulation in *C. apetalus* and *C. quitensis* seedlings could be one of the mechanisms responsible for MSA tolerance in the evaluated species. According to Cuba-Díaz et al. [17] similar effects occurred for high concentrations of copper (II) ions, which induced proline accumulation in the leaves and stems of *C. quitensis*. In plants, proline accumulation is an adaptive mechanism to environmental stress [49]. Karolewski [50] reported on the accumulation of proline in the leaves of *Populus* 'Robusta' plants under exposure to SO_2 and demonstrated that proline pretreatment prevented injury and chlorophyll loss in leaves. The content of free proline in *Oryza sativa* L. also increased under exposure to SO_2 [51].

Morphophysiological variability of *C. quitensis* seeds and seedlings

The morphophysiological variability of *C. quitensis* plants grown from Antarctic seeds and from greenhouse seeds, such as differences in seed germination capacity and seedling growth, could be attributed to the phenotypic variation of seeds. According to Kellmann-Sopyła et al. [10,19], *C. quitensis* seeds are characterized by somatic polymorphism. *Colobanthus quitensis* seeds harvested in Antarctica [15] are significantly longer, wider, and heavier than greenhouse-grown seeds [10]. The seeds of *C. quitensis* also differ in color (Fig. 8). Antarctic seeds are dark brown (Fig. 8A), whereas greenhouse-grown seeds are light brown (Fig. 8B). Similar observations were made for *Silene monoidea* (Caryophyllaceae) in which black seeds are heavier and germinate early in the growing season, whereas lighter brown seeds germinate throughout the growing season and are more dormant [52].

Somatic seed polymorphism appears in a small number of families, such as Asteraceae, Chenopodiaceae, Caryophyllaceae, Fabaceae, and Poaceae. Seed polymorphism increases the plants' ability to survive in extreme environments [52–55]. This mechanism could be driven by competition for nutrients during seed development, genetic constitution,

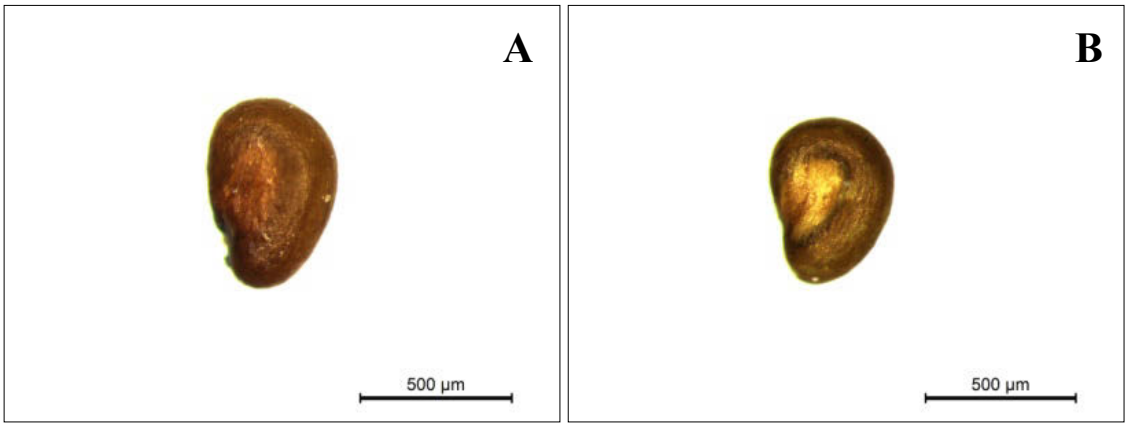

Fig. 8 *Colobanthus quitensis* seeds collected from Antarctica (**A**) and a greenhouse (**B**).

seed maturation and environmental conditions, which influence seed dormancy and germination [52]. Polymorphic seeds differ in mechanical strength and imbibition, which are important determinants of embryonic growth and seed germination rate [56]. Seed size is also correlated with nutrient reserves, and larger reserves contribute to the growth and survival of seedlings [57]. Harper [58,59] observed that polymorphic seeds also differed in germination capacity. In *C. quitensis* plants grown from Antarctic seeds and greenhouse seeds, differences in seed germination capacity and seedling growth were also observed under exposure to salinity, drought, and sodium fluoride stress (Koc et al. data not published). *Colobanthus quitensis* seeds of different size, shape, mass, and color were characterized by various limits of tolerance to MSA treatments. Our data suggest that somatic seed polymorphism is a very important adaptive strategy in *C. quitensis* that maximizes its ability to germinate in harsh environments.

Conclusions

Colobanthus apetalus and *C. quitensis* develop physiological adaptations and are highly resistant to MSA stress. In our study, seed germination capacity in both *Colobanthus* species was high under exposure to high MSA concentration, and MSA did not completely suppress seedling growth. Our results demonstrated that the root systems of both *Colobanthus* species were more sensitive to MSA stress. In seedlings, proline accumulation could be a tolerance mechanism that protects plants against MSA. In *C. quitensis*, somatic seed polymorphism is also a very important adaptive strategy. Other symptoms of exposure to MSA, such as chlorosis and decreased intensity of chlorophyll fluorescence in cotyledons, could be indicative of stress. Interestingly, *C. apetalus* seeds were characterized by the highest germination capacity and germination rate, whereas *C. quitensis* seedlings were more resistant to MSA. In our study, *C. quitensis* seedlings were characterized by more favorable growth and development under MSA stress than *C. apetalus* seedlings. *Colobanthus quitensis* plants generally produced the longest hypocotyls and radicles at the tested MSA concentrations. Methanesulfonic acid also induced a "smaller drop" in the intensity of chlorophyll fluorescence in *C. quitensis* seedlings. The results of this study suggest that *C. quitensis* has developed more effective adaptive mechanisms, which contribute to the species survival in Antarctica, than *C. apetalus* that grows in a more favorable subantarctic condition.

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