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Hormonal and physiological changes driven by fungal endophytes increase Antarctic plant performance under UV-B radiation

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ABSTRACT

Antarctic environments are amongst the most stressful habitats for life on Earth, with high intensities of solar UV-B radiation reaching the land surface. In this study, we evaluated how the photochemical efficiency, cell damage and reproductive biomass of Antarctic pearlwort (*Colobanthus quitensis*) were affected by different intensities of UV-B radiation in the absence and presence of fungal endophytes. In addition, we evaluated the hormonal content of plants at different UV-B radiation intensities and how hormonal content was affected by endophytes. Overall, plants exposed to UV-B radiation showed higher numbers of flowers, higher total biomass and lower lipid peroxidation in the presence of endophytes compared with plants without endophytes. Photochemical efficiency was unaffected. Fungal endophytes affected the content of salicylic acid, jasmonate, indole-3-acetate and abscisic acid in shoot tissue of plants exposed to UV-B radiation. These results suggest that endophytes could modulate the hormonal content of *C. quitensis* to improve its ecophysiological performance under high UV-B radiation.

1. Introduction

Plants are sessile organisms that need to adapt to changing environments and stress conditions, which can limit their growth and development. To deal with extreme environments, plants may interact with micro-organisms in symbiotic relationships, which confer beneficial effects on fitness and evolution to both partners (Rodriguez et al., 2009). The Antarctic continent is considered to be one of the most stressful environments on Earth for plant life (Convey et al., 2014; Pointing et al., 2015). Extreme conditions such as low temperatures, strong winds, high radiation, and low water and nutrient availability affect plant establishment and survival (Robinson et al., 2003; Convey, 2011). Despite these unfavourable and aggressive environmental conditions, two vascular plant species (*Colobanthus quitensis* and *Deschampsia antarctica*) occur in the sub- and maritime Antarctic. *C. quitensis*, commonly known as Antarctic pearlwort, is a small-sized cushion-like perennial herb that is self-compatible (Convey, 1996). This species displays a wide distribution, ranging from Mexico to the Antarctic Peninsula, which has been attributed to its high degree of phenotypic plasticity in ecophysiological traits (Molina-Montenegro et al., 2012; Acuña-Rodríguez et al., 2017).

The ecophysiological performance of Antarctic plants is not only affected by abiotic factors, but also by biotic interactions (Torres-Díaz et al., 2016). In this regard, fungal endophytes are ubiquitous plant symbionts that can strongly influence plant physiological performance under stressful conditions (Giauque and Hawkes, 2013). They can confer fitness benefits to host plants, including tolerance to herbivory, heat, salt and drought and other stress factors, as well as improving nutrient uptake from soil (Arnold et al., 2003; Waller et al., 2005). For example, root-endophytes isolated from *C. quitensis* were recently used to inoculate lettuce

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plants, enhancing their ecophysiological performance and yield under both normal and reduced irrigation (Molina-Montenegro et al., 2016). In addition, Torres-Díaz et al. (2016) showed that Antarctic endophytes improve the ecophysiological performance and fitness related-traits in *C. quitensis* under drought and well-watered conditions. However, despite their known potential to drive the ability of plants to cope with stressful environmental conditions, little is known about whether the endophyte consortium in host plants modulates plant hormonal content.

Exposure to solar radiation, and especially ultraviolet-B (UV-B) radiation (280–315 nm), is an important factor for the growth and performance of Antarctic plants. This portion of the electromagnetic spectrum is a minor component of total solar radiation but carries substantially more energy per photon than radiation of longer wavelengths. Exposure to UV-B radiation has received scientific attention in the last few decades because of the depletion of stratospheric ozone – a natural shield to this short wavelength radiation – above Antarctica (De Laat et al., 2010). It is well known that UV-B radiation damages DNA, inhibits photosynthesis and arrests the cell cycle (Rizzini et al., 2011). Deleterious effects of UV-B radiation on plants take place at high doses in non-acclimated plants and low UV-B radiation doses cause morphogenic effects, such as the inhibition of hypocotyl development, stem and leaf elongation, and leaf thickening (Jansen, 2002).

Hormones control complex molecular programs in plants, modulating for example, responses to biotic and abiotic stresses including exposure to UV-B radiation (Bandurska and Cieslak, 2013). Several studies have been conducted in order to try to elucidate the hormonal profiles of plants in response to UV exposure (Jansen, 2002; Izaguirre et al., 2003; Peng and Zhou, 2009; Tossi et al., 2009; Bandurska and Cieslak, 2013; Dinh et al., 2013; Esringu et al., 2016). In the case of auxin, plants exposed to UV-B radiation show a dwarf phenotype, smaller and thicker leaves with short petioles, short inflorescences and an increased root/shoot ratio, which are all symptoms associated with auxin-regulated processes (Jansen, 2002). The relationship between abscisic acid (ABA) and UV-B exposure has been extensively studied under drought (Bandurska and Cieslak, 2013). ABA is accumulated in response to moderate and high UV-B levels in several species such as lettuce (Esringu et al., 2016), soybean (Peng and Zhou, 2009), tobacco (Dinh et al., 2013) and maize (Tossi et al., 2009). Jasmonate (JA) is well-known as the major plant hormone involved in defence against herbivores, necrotrophs and in wounding responses. Solar UV-B radiation has been reported to increase JA biosynthesis in *Nicotiana* sp. (Izaguirre et al., 2003; Dinh et al., 2013). In addition, salicylic acid (SA), which is associated with defence against biotrophs, is related to the presence of reactive oxygen species (Herrera-Vasquez et al., 2015).

With respect to endophytes, these micro-organisms obtain nutrients by interactions with plants, using strategies to colonise and establish mutualistic relationships with their host (Shen et al., 2006). To date, little information is available about the mechanisms employed by endophytes to modulate the phytohormone contents of their host plants to avoid the plant immune response and maintain the mutualism (Jacobs et al., 2011; Qiang et al., 2011). Little more is known about the ways in which endophytes promote plant root growth, leading to improved nutrient acquisition by their host (Sirrenberg, 2007; Eaton et al., 2011). With the exception of the study by Newsham et al. (1998), who reported a decrease in the numbers of seeds and spikes on *Lolium perenne* plants grown with the leaf endophyte *Neotyphodium lolii* in the presence of UV radiation, little is also known about how endophytes and UV-B radiation interact to determine plant performance.

The main goal of the present study was to identify changes in hormonal profiles underlying the photo-protective effect of endophytes in *C. quitensis*, by measuring plant photochemical efficiency, lipid peroxidation and reproductive biomass under a UV-B radiation gradient. The results provide novel insights into the hormonal and physiological mechanisms triggered by endophytes in their host plants, which translate into improved plant tolerance to the extreme environmental conditions prevailing in Antarctica.

2. Materials and methods

2.1. Plant material and study site

Fifty individuals of *C. quitensis* were collected in the maritime Antarctic from around the Henryk Arctowski base on King George Island, South Shetland Islands (62°09′S; 58°27′W) during the 2015–2016 growing season. The study site corresponds to a fringe parallel to the coast-line on the western shore of Admiralty Bay. The vegetation in this coastal area is typical of maritime Antarctic ice-free soils, and is dominated mainly by the native flowering plant species *D. antarctica* and *C. quitensis*, as well as by mosses and lichens.

2.2. Plant growth, endophyte-free plant production and infection determination

To assess the effects of fungal endophytes on UV-B protection in *C. quitensis*, a manipulative experiment was conducted using endophyte-free plants (hereafter, E-plants) and plants with a consortium of endophytes (hereafter, E+ plants), growing in sterilized Antarctic soil. Endophyte-free plants were obtained by disinfecting E+ plants collected from the study site. Plant samples were transported to the laboratory and planted in 300 ml plastic pots filled with soil from the study site and maintained for 1 month in a climatic chamber at 4 °C with a photon flux density (PFD) of 380 μmol m^{-2} s^{-1} and a 20/4h light/dark photoperiod, mimicking summertime maritime Antarctic climatic conditions.

The commercially available fungicide Benlate (containing benomyl [methyl [1-butylamino carbonyl]-1H-benzimidazol-2-yl] carbamate, DuPont, Wilmington, DE, USA) was used to remove fungal endophyte infection. Benomyl was chosen because no phytotoxic effects have been detected on other plant species, including *C. quitensis* (Spiering et al., 2005; Torres-Díaz et al., 2016). Leaves and roots of *C. quitensis* were completely submerged in tap water containing 2 g l^{-1} of Benlate and maintained for 1 h at room temperature. Each plant of *C. quitensis* was then transplanted to pots (50 cm^3) filled with autoclaved soil (2 h at 140 °C) taken from the study site. To reinforce the removal treatment of fungal endophytes, seedlings were sprinkled with Benlate solution (2 g l^{-1}) once per week for 3 weeks. After 4 weeks of growth, newly emerged leaves were examined microscopically to evaluate the success of Benlate in eliminating endophytes. E+ plants were also treated, but only with water.

Fungal infection of plant tissues was determined by light microscopy and culture-based methods (Bacon and White, 2000). It has been reported that some of the most frequent fungal endophytic genera in *C. quitensis* are *Penicillium, Alternaria* and *Geomyces* (Molina-Montenegro, unpublished data). Infection status was initially checked microscopically on a subset of at least 10% of the plants ($n = 10$). Endophyte occurrence in tissues was quantitatively determined by counting aniline blue-stained endophyte hyphae in leaf cross-sections. This method is a reliable and direct measure of the amount of viable endophyte mycelium (Spiering et al., 2005). Secondly, after Benlate treatment, endophyte-free plants were submitted to re-isolation protocols using culturing-based methods described previously by Bacon and White (2000). The samples of plant tissue that showed no outgrowth of fungi into the

surrounding solid media were considered clean or endophyte-free plants suitable for use in the subsequent experiments.

2.3. Assessing the effect of endophytes on plant ecophysiology and protection mechanisms

To evaluate the effects of fungal endophytes on the ecophysiological performance (photochemical efficiency of PSII, peroxidation of lipids and reproductive biomass) of *C. quitensis* under UV-B exposure, we performed an experiment under controlled conditions in growth chambers over 3 months. A total of 72 individuals were randomly assigned to six treatments: *1 & 2)* with and without endophytes and without UV-B radiation (control treatments, E+ 0 $\mu W\ cm^{-2}$ and E- 0 $\mu W\ cm^{-2}$, respectively), *3 & 4)* with and without endophytes and low level of UV-B (E+ 5.0 $\mu W\ cm^{-2}$ and E- 5.0 $\mu W\ cm^{-2}$, respectively), and *5 & 6)* with and without endophytes and high level of UV-B (E+ 30 $\mu W\ cm^{-2}$ and E- 30 $\mu W\ cm^{-2}$, respectively).

To generate the different UV-B treatments, plastic pots with plants were exposed to 5.0 $\mu W\ cm^{-2}$ and 30.0 $\mu W\ cm^{-2}$ of ultraviolet-B radiation (UV-B; 280–320 nm) in a climatic chamber at 4 °C, with a photoperiod of 20/4 light/dark (380 μmol of photons $m^{-2}\ s^{-1}$). The UV-B conditions were provided by three fluorescent tubes (PL-L 36W/01/4P UV-B; New Jersey, Hammond In, USA) placed 25 cm from each plant and the intensity of UV-B radiation was recorded with a UV light meter (Sentry ST-513, Taiwan, China). The tubes were covered with Mylar film (Du-Pont), which absorbs radiation of wavelengths less than 320 nm. The plants under a single layer of Mylar film received 5.0 $\mu W\ cm^{-2}$. This treatment was considered to be close to natural conditions, since this intensity is that which comes from the average UV-B radiation reported in the literature for Antarctica (Day et al., 2001). In addition, we exposed plants without layers of Mylar filter, generating a higher UV-B intensity (30.0 $\mu W\ cm^{-2}$). Finally, a set of plants (E+ and E-) was exposed under lamps covered with three layers of Mylar film, which absorbed all UV-B radiation, which was considered to be the control condition.

Photochemical efficiency of PSII (Fv/Fm; where Fv = [Fm − F0], Fm = maximum fluorescence yield, and F0 = minimum fluorescence yield) (Maxwell and Johnson, 2000) was estimated using a pulse modulated-amplitude fluorimeter (FMS 2, Hansatech, Instrument Ltd, Norfolk, UK). We used Fv/Fm as a response variable because it correlates with plant fitness and has proven to be a good proxy for the photosynthetic system of a plant (see Molina-Montenegro et al., 2013). To compare the photochemical efficiency between treatments, a group of leaves from each individual was dark-adapted for 30 min (to obtain open PSII centers) using a black-box (30 × 20 × 15 cm) to ensure maximum photochemical efficiency. To assess whether the presence of endophytes was involved in the protection of cell damage, we also measured the oxidative degradation of lipids. Lipid peroxidation was estimated by measuring the concentration of malondialdehyde (MDA) by the thiobarbituric acid (TBA) assay (Egert and Tevini, 2002). At the end of the experiment, fresh tissue (0.1 g) of *C. quitensis* individuals submitted to UV-B treatments were homogenized with 2 ml of TCA (1%) and centrifuged at 10,000*g* for 5 min. 250 ml of the supernatant was mixed with 1 ml of TBA (0.5%) in TCA (20%). Mixtures were incubated in boiling water for 30 min, and then cooled to room temperature. Absorbance was determined at 532 nm and non-specific absorbance at 600 nm (Hodges et al., 1999). The MDA content was determined using a molar extinction coefficient of 155 $mol^{-1}\ cm^{-1}$. Finally, reproductive biomass was assessed as the difference between total fresh reproductive (floral) biomass at the start and end of the experiment. All these measurements (photochemical efficiency of PSII, peroxidation of lipids and reproductive biomass) were taken at the end of the experiment, which lasted for 3 months.

All treatments were performed in three automatic air-cooling growth chambers (model: LTJ300LY; Tianyi Cool, China) running at 4 °C. All six treatments were applied inside each chamber. Since treatments can be affected by the characteristics of the growth chamber (see Potvin and Tardif, 1988), individuals from different treatments were randomly moved within growth chambers every week. At the end of each month, the growth chambers were switched off, cleaned, and individuals were transferred between chambers, maintaining all treatments in each chamber and growth conditions were re-established.

2.4. Hormone profile analysis

Plants from different treatments (total $n = 12$) were harvested after 3 months. Shoot and root tissue were separated and were immediately frozen in liquid nitrogen and stored at −80 °C until lyophilization. Plant hormone extraction was carried out as previously described (Carrasco Loba and Pollmann, 2017; Carrasco Loba et al., 2017). In brief, 1 ml of pre-warmed (65 °C) methanol was added to approximately 50 mg of each lyophilized plant sample and the extraction proceeded for another 60 min at room temperature with gentle shaking. Each sample was supplemented with 37 pmol [$^2H_6$]-ABA, 50 pmol [$^2H_2$]-IAA, 50 pmol [$^2H_4$]-SA, and 50 pmol [$^2H_5$]-JA (stable isotope-labelled internal standards). Cell-free supernatants were dried under vacuum and pre-purified for subsequent gas chromatography-mass spectrometry analysis. For this, the dry extracts were fully dissolved in 50 μl methanol and 200 μl of diethyl ether. Thereafter, they were loaded onto aminopropyl solid-phase extraction cartridges. Each cartridge was washed twice with 250 μl of $CHCl_3$:2-propanol (2:1, v/v) before the hormone-containing fraction was eluted with 400 μl of acidified diethyl ether (2% acetic acid, v/v). The eluates were transferred into 0.8 ml capacity autosampler vials and again taken to dryness in a gentle stream of nitrogen. Prior to mass spectrometric assessment, samples were derivatized by adding 20 μl of a mix consisting of 220 μl of acetone:methanol (9:1, v/v), 27 μl of diethyl ether and 3 μl of a (trimethylsilyl)diazomethane solution (2.0 M in diethyl ether) and letting them rest for 30 min at room temperature. The settings for the gas chromatograph and the mass spectrometer were as described previously (Carrasco Loba et al., 2017). The following transitions were recorded: MeSA, *m/z* 152 to *m/z* 120 and *m/z* 120 to *m/z* 91; [$^2H_4$]-MeSA, *m/z* 156 to *m/z* 124 and *m/z* 124 to *m/z* 95; MeJA, *m/z* 151 to *m/z* 108 and *m/z* 224 to *m/z* 151; [$^2H_5$]-MeJA, *m/z* 154 to *m/z* 111 and *m/z* 229 to *m/z* 154; MeABA, *m/z* 162 to *m/z* 133 and *m/z* 134 to *m/z* 90; [$^2H_6$]-MeABA, *m/z* 168 to *m/z* 139 and *m/z* 140 to *m/z* 90; MeIAA, *m/z* 189 to *m/z* 130 and *m/z* 130 to *m/z* 103; [$^2H_2$]-MeIAA, *m/z* 191 to *m/z* 132 (quantifier ion) and *m/z* 132 to *m/z* 103 (qualifier ion), respectively in all determinations. The amount of endogenous hormone content was calculated from the signal ratio of the unlabelled over the stable isotope-containing mass fragment observed in the parallel measurements.

2.5. Statistical analysis

Data obtained from three individual experiments were evaluated using two-way ANOVA-LSD tests to determine the main and interactive effects of UV radiation and endophytes for each determination. Tukey's pairwise comparisons test was used to compare individual means. All analyses were performed with STATISTICA for Windows (v. 7.0; StatSoft, Tulsa, OK, USA). Significant differences were inferred at $P \leq 0.05$.

3. Results

3.1. Ecophysiological performance and protective mechanism induced by endophytes in C. quitensis exposed to UV-B radiation

Assessment of the photochemical efficiency of PSII revealed that this physiological parameter was not affected by the presence of endophytes in control plants, nor in plants exposed to different intensities of UV-B radiation compared to those plants without endophytes (Fig. 1). The interaction between UV-B radiation and the presence of endophytes did not affect the photochemical performance of plants (Table 1). Regarding the cell damage provoked by UV-B radiation exposure, plants without endophytes displayed significantly higher contents of thiobarbituric acid reactive substances (TBARS), which is indicative of lipid peroxidation. The data indicated that interaction between both factors did not significantly affect cell damage, but that the presence of endophytes and UV-B radiation affected lipid peroxidation (Table 1). In plants with endophytes and exposed to UV-B radiation, lipid peroxidation was significantly increased at 5 μW cm^{-2} and 30 μW cm^{-2} compared to the control. However, compared to plants without endophytes, no significant differences were observed between the two groups at 0 or 5.0 μW cm^{-2}, though at 30 μW cm^{-2} of radiation exposure, plants with endophytes had significantly less TBARS (Fig. 1).

Total and reproductive biomass analyses showed significant differences between treatments in plants without endophytes (E -) exposed to UV-B radiation at both higher intensities compared with the control plants, decreasing both parameters. The interaction between UV-B radiation and the presence of endophytes significantly affected the total flower number (Table 1). The presence of endophytes increased flower number by almost two-fold compared with the same treatment with no endophytes (Fig. 2), and statistical

Table 1
Results of factorial ANOVAs evaluating the interactive effects of endophytes presence (E) and UV-B radiation treatments (low radiation (5.0 μW cm^{-2}), high radiation (30 μW cm^{-2}) and controls (0 μW cm^{-2})) on photochemical performance, measured as maximum quantum yield (Fv/Fm) of photosystem II (PSII), total biomass (mg), flowers number and lipid peroxidation by TBARS (mmol mL^{-1}/g FW) of *Colobanthus quitensis*. Significant *P* values ($P < 0.05$) are highlighted in bold.

Source of variation	d.f.	MS	F	P
Photochemical efficiency (Fv/Fm)				
UV treatments (UV)	2	0.0007473	3.4740	**0.0381**
Endophytes presence (E)	1	0.0001441	0.6702	0.4166
UV x E	2	0.0000352	0.1634	0.8497
Error	54	0.0002151		
TBARS (mmol mL^{-1}/g FW)				
UV treatments (UV)	2	4.326	32.03	**<0.0001**
Endophytes presence (E)	1	1.233	9.126	**0.0038**
UV x E	2	0.3407	2.522	0.0897
Error	54	0.1351		
Flowers number				
UV treatments (UV)	2	106	86.34	**<0.0001**
Endophytes presence (E)	1	854.3	695.5	**<0.0001**
UV x E	2	5.527	4.500	**0.0156**
Error	54	1.228		
Total biomass (mg)				
UV treatments (UV)	2	0.2489	69.76	**<0.0001**
Endophytes presence (E)	1	0.2233	52.58	**<0.0001**
UV x E	2	0.0859	23.43	**<0.0001**
Error	54	0.0036		

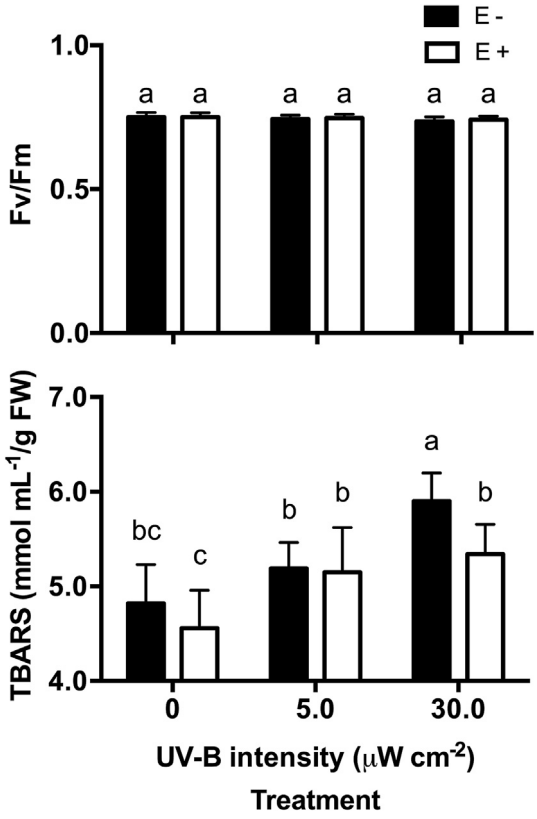

Fig. 1. Ecophysiological performance (photochemical efficiency - Fv/Fm and lipid peroxidation) in individuals of *Colobanthus quitensis* exposed to UV-B radiation with and without endophytes. Individuals were exposed to 0, 5.0 and 30 μW cm^{-2} UV-B radiation intensities. Black bars correspond to plants without endophytes (E -) and white bars to plants with endophytes (E +). Different letters indicate significant differences ($P < 0.05$; Tukey-test). Bars represent means ± S.E. (n = 10).

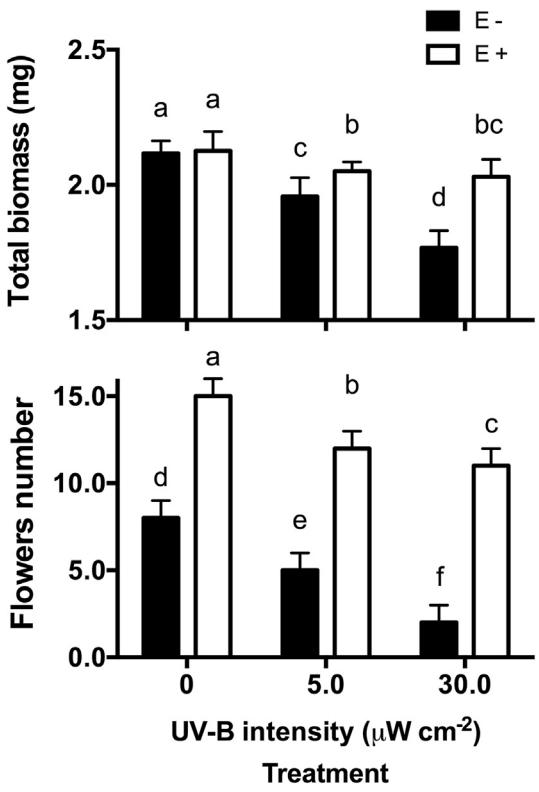

Fig. 2. Fitness performance (flowers number and total biomass) of *Colobanthus quitensis* exposed to UV-B radiation with and without endophytes. Individuals were exposed to 0, 5.0 and 30 μW cm^{-2} UV-B radiation intensities. Black bars correspond to plants without endophytes (E -) and white bars to plants with endophytes (E +). Different letters indicate significant differences ($P < 0.05$; Tukey-test). Bars represent means ± S.E. (n = 10).

analysis indicated a significant effect of endophytes on reproductive biomass (Table 1). In the case of total biomass, UV-B radiation induced a significant decrease in a dose-dependent manner, whereas in E + plants this decrease was statistically less compared with E − plants (Fig. 2).

3.2. Fungal endophytes modulate plant hormone content in shoot tissue depending on UV-B radiation exposure

Hormone content analysis revealed that UV-B treatment increased the foliar salicylic acid (SA) content in plants without endophytes, however, higher levels of UV-B radiation did not further increase salicylic acid content (Fig. 3). The statistical analysis indicated an interactive effect of UV-B radiation and endophytes on endogenous SA content (Table 2). Nevertheless, SA content was significantly lower with endophytes and did not change with or without UV-B radiation treatment (Fig. 3).

In the case of jasmonic acid (JA), only high UV-B radiation exposure (30 $\mu W\,cm^{-2}$) significantly increased JA content, but no differences in JA content were observed between 0 $\mu W\,cm^{-2}$ and 5.0 $\mu W\,cm^{-2}$ without endophytes. Statistical analysis indicates that the interaction of both factors showed an additive effect, decreasing the JA levels, but that UV-B radiation alone was not a significant factor (Table 2). However, at 0 $\mu W\,cm^{-2}$ of UV-B radiation, JA content did not alter in seedlings with and without endophytes. In contrast to the observations made in the absence of endophytes, plants with endophytes showed a significant decrease of JA content in seedlings exposed to 5.0 $\mu W\,cm^{-2}$ and 30 $\mu W\,cm^{-2}$ (Fig. 3).

Foliar contents of the major auxin in plants, indole-3-acetic acid (IAA), did not show significant differences elicited by endophytes at 0 $\mu W\,cm^{-2}$ of UV-B radiation, but under radiation of 5.0 $\mu W\,cm^{-2}$ and 30 $\mu W\,cm^{-2}$, the content of IAA was reduced in seedlings with endophytes compare to those without (Fig. 3). Both UV-B radiation and endophyte presence significantly affected the IAA content in shoot tissue of *C. quitensis* (Table 2).

Table 2
Results of factorial ANOVAs evaluating the interactive effects of endophytes presence (E) and UV-B radiation treatments (low radiation (5.0 $\mu W\,cm^{-2}$), high radiation (30 $\mu W\,cm^{-2}$) and control (0 $\mu W\,cm^{-2}$)) on salicylic acid, jasmonic acid, indol-3-acetic acid and abscisic acid concentrations (pmol/g FW) of *Colobanthus quitensis* shoot tissue. Significant *P* values (<0.05) are highlighted in bold.

Source of variation	d.f.	MS	F	P
Salicylic acid content (pmol/g FW)				
UV treatments (UV)	2	24.12	178.8	**<0.0001**
Endophytes presence (E)	1	393.1	2914	**<0.0001**
UV x E	2	29.95	222.1	**<0.0001**
Error	12	0.1349		
Jasmonic acid content (pmol/g FW)				
UV treatments (UV)	2	0.00005411	0.281	0.7599
Endophytes presence (E)	1	0.04115	213.7	**<0.0001**
UV x E	2	0.00847	43.99	**<0.0001**
Error	12	0.0001926		
Indol-3-acetic acid content (pmol/g FW)				
UV treatments (UV)	2	62.53	436.4	**<0.0001**
Endophytes presence (E)	1	24.56	171.4	**<0.0001**
UV x E	2	2.087	14.57	**0.0006**
Error	12	0.1433		
Abscisic acid content (pmol/g FW)				
UV treatments (UV)	2	2741	88.95	**<0.0001**
Endophytes presence (E)	1	34.78	1.129	0.309
UV x E	2	1234	40.06	**<0.0001**
Error	12	30.81		

Finally, abscisic acid (ABA) content was higher in control seedlings without endophytes compared to those with endophytes (Fig. 3). Under 5.0 $\mu W\,cm^{-2}$ of UV-B radiation, ABA content in plants with and without endophytes increased compared to plants exposed to 0 $\mu W\,cm^{-2}$ of UV-B radiation, not showing significant differences between them. The statistical analysis indicated that the interaction between both showed a statistical significant effect (Table 2). However, the content of ABA was lower in seedlings with endophytes at high UV-B radiation compared to the plants without endophytes (Fig. 3).

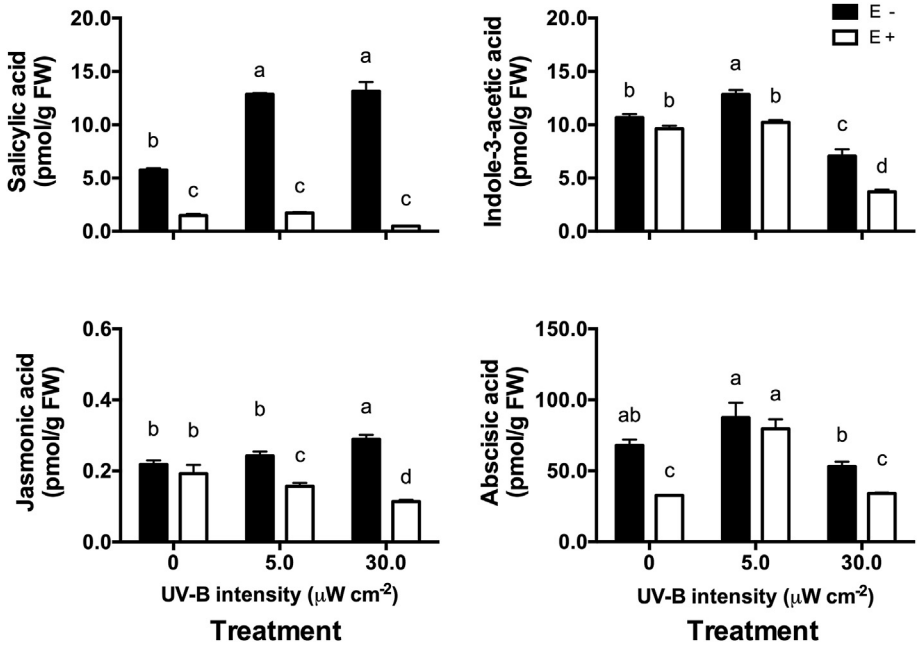

Fig. 3. Content of hormones in shoots of plants exposed to UV-B radiation with and without endophytes. *Colobanthus quitensis* individuals exposed to 0, 5.0 and 30 $\mu W\,cm^{-2}$ UV-B radiation intensities were evaluated. Black bars correspond to plants without endophytes (E -) and white bars to plants with endophytes (E +). Different letters indicate significant differences ($P < 0.05$; Tukey-test). Bars represent means ± S.E. (n = 6).

4. Discussion

Plants are constantly exposed to several environmental factors that affect their performance. Solar radiation is one of the most relevant factors in the natural environment, since it has prominent impacts on a number of processes, such as leaf and root development, seed germination and flowering. UV-B radiation represents, under normal conditions, a small fraction of solar radiation. However, due to the depletion of stratospheric ozone, intense UV-B radiation reaches Antarctica (De Laat et al., 2010). In this extreme environment, endophytes provide benefits to resident vegetation that is continuously exposed to harsh conditions like nutrient deficiency, drought, extreme cold and high UV-B radiation (Rodriguez et al., 2009). Previous studies demonstrated that the inoculation of roots of agriculturally important crop plants with endophytes isolated from Antarctica translates into increased fitness under normal and reduced irrigation regimens (Molina-Montenegro et al., 2016). For these reasons, the study of native vegetation with their associated micro-organisms that grow under extreme UV-B radiation conditions is very important for understanding the mechanism(s) facilitating plant survival in the natural environment.

The effects of high UV-B radiation doses on plants are generally due to an overproduction of free radicals, which, among other mechanisms, induces cell damage by lipid peroxidation (Du and Jin, 2000). UV-B radiation delays the flowering of several species, but the molecular mechanism behind this process is not well understood (Dotto and Casati, 2017). Our results suggest that the presence of endophytes could confer tolerance to high UV-B radiation in *C. quitensis* and physiological improvements, evidenced by decreased cell damage, less lipid peroxidation and an increase in fresh weight of total and reproductive biomass. In addition, we found that endophytes significantly increased flower number, and that UV-B radiation significantly reduced flower number, and that endophytes and UV-B interacted to determine this parameter and also total biomass. These fitness parameters were positively affected by the presence of endophytes in plants exposed to UV-B radiation. These results may be due to changes in the concentrations of hormones in leaf tissue, as was observed for SA. This significant increase in reproductive biomass observed in plants with endophytes is in accordance with previous studies on *Arabidopsis* SA-deficient transgenic lines and mutants, where the analysis of seed yield revealed an improvement in plants with less SA content compared to the wild type plants with more SA content (Abreu and Munné-Bosch, 2009). Also, a previous report in tall fescue colonised by fungal endophytes showed an increase in total biomass, but a decrease in reproductive biomass after burn treatment, indicating that endophytes can affect the reproductive biomass of host plants (Hall et al., 2014). This hormone modulation triggered by endophytes could help *C. quitensis* to cope with the stressful conditions encountered in the Antarctic and possibly with climate warming.

An interesting relationship seems to exist between endophyte symbiosis and JA levels. The content of JA was decreased in plants with endophytes, which may be associated with a previous report where a JA biosynthesis mutant reduces flowering and induces resistance against pests (Chehab et al., 2012). In addition to the observations made for JA, ABA contents increase in plants with endophytes. This can be attributed to the role of ABA signalling mediated by the transcription factor ABSCISIC ACID-INSENSITIVE 4 (ABI4), which delays flowering by negative regulation of *FLOWERING LOCUS C* (*FLC*) transcription (Shu et al., 2016). Also, a relation between ABA and a UV-B protection mechanism has been proposed to be regulated by the MYB4 transcription factor that orchestrates flavonoid accumulation, which in turn leads to increased UV-B photo-protection (Zhao et al., 2007). MYB4 imported into the nucleus by a nuclear importin transporter has been reported to be sensitive to ABA and drought 2 (SAD2) (Zhao et al., 2007).

SA is known to increase during plant defence response and under UV stress conditions in several species including tobacco (Yao et al., 2011), *Arabidopsis* (Mintoff et al., 2015), and pepper (Mahdavian et al., 2008). Navarro-Meléndez and Heil (2014) showed a reduction in SA contents in leaves of lima beans infected with symptomless endophytes. Additionally, Bastías et al. (2018) showed that symbiotic plants display lower concentrations of SA compared with the nonsymbiotic counterpart, proposing an anti-herbivore mechanism conferred by the fungal endophytes. Previously, Bastías et al. (2017) report a revised understanding of the relationship between JA and SA modulation in endophytes-symbiotic plants and the plant immune system. These antecedents agreed with the observations in *C. quitensis*, which are suggestive of an activation of protective mechanisms against UV-B radiation that may be mediated by the plant immune system based on the observed modulation of phytohormones.

Regarding the IAA content, UV-B radiation induced a decrease in the hormone level that agreed with previous observations in the model plant *Arabidopsis* (Hectors et al., 2012). This decrease was higher in plants colonised by endophytes, suggesting that endophytes could be modulating the biosynthesis or/and transport of IAA. It is well known that flavonoid blocks the polar auxin transport (PAT) and specifically flavonols (Yin et al., 2014). We have preliminary results that show an increase in the concentration of quercetin and kaempferol in endophyte-infected plants exposed to high UV radiation (Ramos et al. unpublished). These are in accordance with results of *D. antarctica* plants exposed to UV-B radiation, which display high contents of total phenolic compounds (Köhler et al., 2017). These observations support the hypothesis that endophytes may activate the flavonol biosynthesis pathway, which may reduce IAA content.

Finally, our observations indicate that endophytes improve the physiological performance of *C. quitensis* by reducing cell damage through the modulation of phytohormone levels, which are involved in the control of several plant molecular mechanisms, allowing the endophytes to live in their host in a mutualistic association. This association could be considered as a functional symbiosis that provides benefits to *C. quitensis*, allowing the species to cope with the harsh conditions encountered in Antarctica, promoting reproductive propagation and increasing plant genetic variability.

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