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Acclimation to a combination of water deficit and nutrient deprivation through simultaneous increases in abscisic acid and bioactive jasmonates in the succulent plant *Sempervivum tectorum* L

Sabina Villadangos [a,b], Sergi Munné-Bosch [a,b,*]

[a] *Department of Evolutionary Biology, Ecology and Environmental Sciences, Faculty of Biology, Universitat de Barcelona, Avinguda Diagonal 643, 08028, Barcelona, Spain*
[b] *Institute of Research in Biodiversity (IRBio), Universitat de Barcelona, Avinguda Diagonal 643, 08028, Spain*

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ABSTRACT

Activation of hormonal responses defines the drought acclimation ability of plants and may condition their survival. However, aside ABA, little is known about the possible contribution of other phytohormones, such as jasmonates and salicylates, in the response of CAM plants to water deficit. Here, we aimed to study the physiological mechanisms underlying the stress tolerance of house leek (*Sempervivum tectorum* L.), a CAM plant adapted to survive harsh environments, to a combination of water deficit and nutrient deprivation. We exposed plants to the combination of these two abiotic stresses by withholding nutrient solution for 10 weeks and monitored their physiological response every two weeks by measuring various stress makers together with the accumulation of stress-related phytohormones and photoprotective molecules, such as tocopherols (vitamin E). Results showed that ABA content increased by 4.2-fold after four weeks of water deficit to keep later constant up to 10 weeks of stress, variations that occurred concomitantly with reductions in the relative leaf water content, which decreased by up to 20% only. The bioactive jasmonate, jasmonoyl-isoleucine was the other stress-related phytohormone that simultaneously increased under stress together with ABA. While contents of salicylic acid and the jasmonoyl-isoleucine precursors, 12-*oxo*-phytodienoic acid and jasmonic acid decreased with water deficit, those of jasmonoyl-isoleucine increased 3.6-fold at four weeks of stress. The contents of ABA and jasmonoyl-isoleucine correlated positively between them and with the content of α-tocopherol per unit of chlorophyll, thus suggesting a photoprotective activation role. It is concluded that *S. tectorum* not only withstands a combination of water deficit and nutrient deprivation for 10 weeks without any symptom of damage but also activates effective defense strategies through the simultaneous accumulation of ABA and the bioactive jasmonate form, jasmonoyl-isoleucine.

1. Introduction

Stress-related phytohormones are low-molecular-weight compounds synthesized at very low amounts that activate a signaling response with a consequent physiological action and play an essential role in plant response to stress, which is an unfavorable environmental (abiotic) or biotic condition for growth. Among the various phytohormones described thus far, it is known that, aside from interacting with one another, some of them are especially important to activate defense responses. ABA is one of the best-known phytohormones in this regard since it has been shown to be very effective in modulating key aspects of plant responses to water deficit such as transpiration efficiency, hydraulic conductivity and leaf growth (Thompson et al., 2007; Davies, 2010). Other stress-related phytohormones, such as salicylates and jasmonates, have also been shown to be very effective in the activation of defense strategies to prevent or withstand stress. Better known for their role in acclimation to biotic stresses, the role of salicylates and jasmonates in abiotic stress responses, such as to water deficit, is more slowly but also progressively being revealed, particularly with the progress made during the last two decades (Davies, 2010; Kazan, 2015; Khan et al., 2015; Ilyas et al., 2021).

Together with ABA, jasmonates have been implicated in promoting stomatal closure (Suhita et al., 2004; Munemasa et al., 2007).

* Corresponding author. Department of Evolutionary Biology, Ecology and Environmental Sciences, Faculty of Biology, University of Barcelona, Avinguda Diagonal 643, 08028, Barcelona, Spain.
E-mail address: smunne@ub.edu (S. Munné-Bosch).

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Abbreviations

ABA	Abscisic acid
CAM	Crassulacean acid metabolism
Chl	Chlorophyll
JA	Jasmonic acid
JA-Ile	Jasmonoyl-isoleucine
MDA	Malondialdehyde
OPDA	12-*oxo*-phytodienoic acid
SA	Salicylic acid

Jasmonates have also been shown to be involved in the modulation of root hydraulic conductivity which can contribute to water uptake from soil under water deficit (Sánchez-Romera et al., 2014). Moreover, jasmonates have also been proposed to activate vitamin E biosynthesis in plants (Sandorf and Holländer-Czytko, 2002; Siles et al., 2018; Casadesús et al., 2021), as it occurs with ABA (El Kayal et al., 2006; Chaudhary and Khurana, 2009; Muñoz and Munné-Bosch, 2019). However, whether the jasmonates role in these processes is exerted through ABA-dependent or independent pathways, and specifically which jasmonate forms (either 12-*oxo*-phytodienoid acid [OPDA], jasmonic acid [JA] or its conjugated form, jasmonoyl-isoleucine [JA-Ile]) are the bioactive ones regulating these processes, appear to be less clear and results seem to be influenced by the study model and the experimental approach used (Savchenko et al., 2014; Hewedy et al., 2023).

Crassulacean acid metabolism (CAM) is one of the most important adaptive mechanisms allowing succulent plants to survive in drought-prone environments. Previous studies have shown that ABA can induce CAM metabolism and modulate stomatal closure in several constitutive or facultative species (Taybi et al., 2002; Fleta-Soriano et al., 2015; Cela et al., 2009; Wakamatsu et al., 2021), as well as induce chloroplast clumping (Kondo et al., 2004) and modulate vitamin E accumulation (Fleta-Soriano et al., 2015; Cela et al., 2009), thus playing a role in photoprotection. Unfortunately, however, extraordinarily little is still known about the role of other stress-related phytohormones, such as salicylates and jasmonates in the adaptive responses to water deficit in this interesting group of species. The contents of JA and JA-Ile decreased during the salt-induced transition from C3 to CAM metabolism in the halophyte *Mesembryanthemum crystallinum*, specifically at night, when stomatal closure occurs, thus suggesting stomatal closure is modulated by ABA independently of jasmonates (Wakamatsu et al., 2021). Furthermore, ABA, but not salicylic acid (SA) or JA contents correlated with γ-tocopherol accumulation in water-stressed *Aptenia cordifolia* (Fleta-Soriano et al., 2015; Cela et al., 2009). However, in these latter studies neither OPDA nor JA-Ile, which are generally considered to be bioactive jasmonate forms for several physiological processes (Fonseca et al., 2009; Bosch et al., 2014; De Ollas et al., 2018), were measured, thus potentially masking a jasmonate-mediated effect. Aside from these previous observations, to our knowledge no additional studies have been performed in CAM plants to elucidate the role of stress-related phytohormones other than ABA in their acclimation to water deficit. Furthermore, in drought-prone environments CAM plants usually survive under a combination of water deficit and nutrient deprivation, since drought stress events usually bring together periods of nutrient starvation due to the poor soils where these plants are usually growing (Forseth, 2010; Yu et al., 2017).

Sempervivum tectorum L. (Crassulaceae) is a monocarpic oreophyte CAM plant native from the European high mountains located in the Alps, Pyrenees, and Balkans (Praeger, 1932; Klein and Kaderit, 2015). Besides, it is also widespread through Mediterranean rocky locations where it is naturally distributed (Smith, 1981) and it is also cultivated outside its original range (Parnell and Favarger, 1993). This species can grow well under extreme conditions in its natural habitat surviving up to seven months of snow in winters (Larcher and Wagner, 1983), having its main growing period in spring (May–June) and flowering during summer (July–September). Several studies have previously shown the extreme tolerance of *Sempervivum* species to harsh environments such as sudden frost or heat (Larcher et al., 2010; Zaharia et al., 2010). Unlike its congener *S. montanum*, which is located at the highest altitudes, *S. tectorum* is generally found in south-facing slopes of mountains at lowest altitudes which makes the plant to be particularly exposed to periodic drought events (Larcher et al., 1989). To our knowledge, there is only a previous study showing the physiological response to water stress of this plant species (Glušac et al., 2013), however, the hormonal response was not examined. Furthermore, no studies have examined thus far their response to a combination of water deficit and nutrient deprivation. Here, we aimed to study the physiological mechanisms underlying stress tolerance in *S. tectorum* L., a CAM plant adapted to survive harsh environments, with an emphasis on evaluating the time-course evolution of the contents in endogenous stress-related phytohormones to a combination of water deficit and nutrient deprivation. We hypothesized that not only ABA but also JA-Ile may be involved in the plant stress response in *S. tectorum*, both simultaneously increasing with vitamin E accumulation in leaves.

2. Materials and methods

2.1. Plant material, growth conditions, treatments, and samplings

House leek (*Sempervivum tectorum* L.) individuals, which were grown in 0.3 L-pots of peat:perlite:vermiculite (2:1:1, by volume), were purchased in a local garden (Jardiland S.A., Sant Cugat del Vallès, Spain) and immediately transferred to a greenhouse situated at the Faculty of Biology of the University of Barcelona (Barcelona, NE Spain), where they grew for four additional weeks before experiments started on November 18, 2021. Plants used for the study were selected considering the lack of visual pathogens, the diameter of the rosette and the number of offspring clones, so that all plants were healthy, and their size was very similar (for an example of plant phenotype at the start of experiment, see Fig. 1). Potted plants growing in the greenhouse were exposed to direct natural sunlight but a constant 16h photoperiod throughout the study, which was achieved by using high pressure sodium lamps supplying an additional photosynthetically active photon flux density (PPFD) of ~ 50 μmol m^{-2} s^{-1}. Plants were watered twice a week with half-diluted Hoagland nutrient solution prior to the start of the experimental period.

Two water regimes were imposed on plants at the start of the experiment: well-irrigated control plants, which were kept watered with the same nutrient solution twice a week, and stressed plants, for which irrigation with nutrient solution was withheld during the whole experiment. Samplings were performed at the beginning of the experiment (time 0) and after 2, 4, 6, 8 and 10 weeks of treatments. All samplings were performed at maximum diurnal PPFD at midday (between 11 and 13h solar time). At each sampling point, fully-expanded leaves from the mid part of the mother rosette were used for measurements and immediately frozen in liquid nitrogen to store them later at −80 °C for posterior biochemical analyses. Daily maximum air temperature and relative humidity ranged between 19 and 35 °C and 28–89%, respectively, during the study; and the maximum daily PPFD received by plants was ~ 950 μmol m^{-2} s^{-1}. Therefore, although for simplicity, we will refer here to stressed plants, plants from this treatment were exposed to a combination of water deficit and nutrient deprivation, all in an environmental context characterized by a long photoperiod, and occasional periods of combined abiotic stress due to high temperatures occurring simultaneously with low relative humidity in the air at midday. As all samplings were performed at midday on sunny, clear days, the monitoring of parameters related to the combination of water deficit and nutrient deprivation really reflects a situation of severe abiotic stress that occurs in combination with other environmental

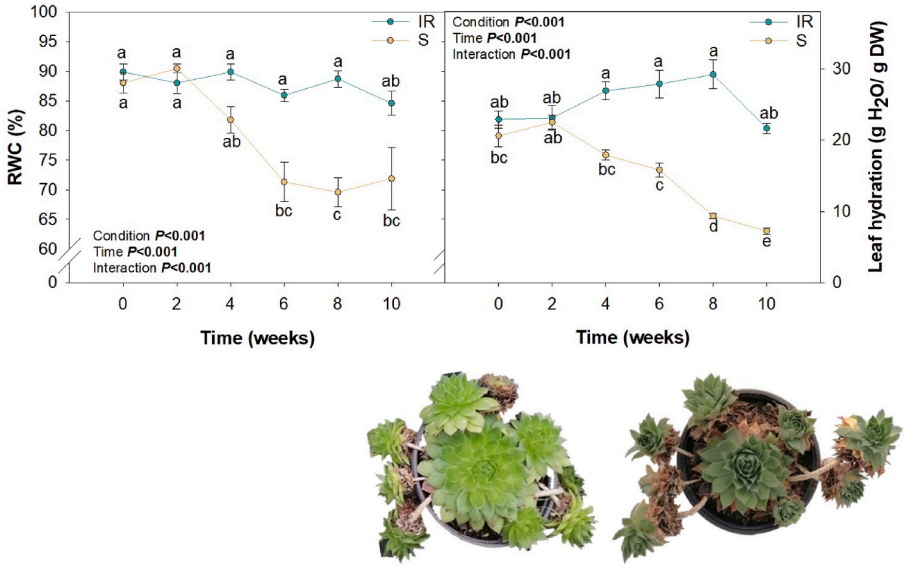

Fig. 1. Leaf water status, estimated as the relative leaf water content (RWC) and leaf hydration (H) of *Sempervivum tectorum* of both irrigated and non-irrigated groups through the stress acclimation process over 10 weeks. *P* values of two-way ANOVAs are shown. Different letters indicate significant differences between treatment conditions and time. Data are mean of n = 8 plants for the irrigated (IR) group and n = 9 plants for the stressed (S) group. As a representative individual from the latter group, the same plant is shown at week 0 (*left*) and week 10 (*right*) from the onset of nutrient solution withdrawal.

stresses at midday.

2.2. Chlorophyll fluorescence, leaf water status and leaf mass

The maximum quantum efficiency of PSII photochemistry (F_v/F_m) was used as a photoinhibition indicator (*sensu* Takahashi and Badger, 2011) and was measured in dark-adapted leaves (at least for 1h) by chlorophyll (Chl) fluorescence using the Mini-PAM II portable fluorimeter Photosynthesis Yield Analyzer (Walz, Germany). The same leaves were then used to estimate the relative leaf water content (RWC) and leaf hydration (H). For that purpose, fresh leaves were weighed to estimate the fresh matter (FW), then immersed in distilled water at 4 °C for 24h to obtain the turgid matter (TW) and oven-dried at 65 °C until constant weight to estimate the dry matter (DW). RWC was calculated as follows: 100 x (FW-DW)/(TW-DW); whereas H was calculated as (FW-DW)/DW. Furthermore, leaf mass was measured by weighing fresh and dried leaves (oven-dried to constant weight as described before) and expressed per g of FW or DW.

2.3. Chlorophyll content

To determine Chl content in leaves, 100 mg of fresh material was ground in liquid nitrogen and extracted in 1.2 mL of cold methanol containing 0.01% of butylated hydroxytoluene. Supernatants were pooled after a three-step extraction, each step consisting of 30 min of cold ultrasonication (Branson 2510 ultrasonic cleaner, Bransonic, Danbury, CT, USA), vortexing and centrifugation at 15.980*g* for 10 min at 4 °C (PrismR, Labnet International Inc.). Then, 200 µl of extracts were diluted 1/4 (v/v) with cold methanol and absorbances were read by UV/Visible spectrophotometry at 470, 653, 666 and 750 nm (CE7400 Aquarius, Cecil Instruments Ltd, Cambridge, UK). Chl *a* and Chl *b* contents were calculated as described by Lichtenthaler and Wellburn (1983).

2.4. Lipid peroxidation analyses

To determine the extent of lipid peroxidation, malondialdehyde (MDA) content was determined spectrophotometrically by using the thiobarbituric acid-reactive-substances assay as described in Hodges et al. (1999). In brief, 100 mg of fresh leaves were ground in liquid nitrogen and extracted with 1 mL of 80% (v/v) cold ethanol with 0.01% (w/v) BHT. A three-step extraction was performed, each step consisting in 30 min of cold ultrasonication, vortexing the extract before, during and after sonication, followed by a 10 min centrifugation at 9402*g* at room temperature. The final extract volume was 3 mL. Then, 1.5 mL of supernatants were pipetted to an eppendorf with 5% (w/v) polyvinylpolypyrrolidone (PVPP), a reagent to reduce cross-reactivity of phenolic compounds that could interfere in the assay. *S. tectorum* is known to have high phenolic content in leaves (Abram and Donko, 1999), being the reason why PVPP was needed in the protocol. After vortexing and a 10 min centrifugation, 0.75 mL of supernatant + PVPP were incubated with -TBA solution and another 0.75 mL were incubated with +TBA solution in glass tubes during 25 min at 95 °C. Then, the reaction was stopped by placing the tubes at 4 °C for 10 min and were centrifugated for 5 min at 9402 *g* at room temperature. Finally, 200 µL of each sample were pipetted per triplicate in a 96-well plate and absorbances were read spectrophotometrically at 440, 532, 600 and 800 nm, after which MDA concentrations were quantified following the equations described by Hodges et al. (1999).

2.5. Tocopherols analyses

Quantification of tocopherols (vitamin E) was performed by high-performance liquid chromatography (HPLC) as described by Amaral et al. (2005). From the previous methanolic extracts detailed above (section 2.3.), 250 µL were transferred into vials by passing through hydrophobic 0.45 µm PTFE filters (Phenomenex, Torrance, CA, USA) and injected in the HPLC system. The HPLC system consisted on a Waters 600 controller pump, a Waters 717 plus auto-sampler and a Jasco FP-1520 fluorescence detector). Tocopherols were separated by using a mobile phase of *n*-hexane and 1,4-dioxane (95.5:4.5, v/v) at a flow rate of 0.7 mL/min using an Inertsil 100A, 5 µm, 250 × 3.0 mm column (GL Sciences, Torrance, CA, USA). Fluorescence was measured with an excitation at 295 nm and emission at 330 nm. A calibration curve with standards of α-tocopherol and γ-tocopherol (Sigma-Aldrich, Steinheim, Germany) was established for correct quantification.

2.6. Stress-related phytohormones analyses

Endogenous contents of stress-related phytohormones, including ABA, the jasmonates OPDA, JA and JA-Ile, and SA were determined by using ultrahigh-performance liquid chromatography (UHPLC) coupled to tandem mass spectrometry (MS/MS) as previously described by Morales et al. (2015). Briefly, 200 µL of methanolic extracts containing deuterium-labeled internal standards were passed onto 0.22 µm PTFE filters (Phenomenex, Torrance, CA, USA) in vials and were injected into

the UHPLC-MS/MS system, consisting in an HPLC coupled to a triple quadrupole mass spectrometer (QTRAP 4000, AB Sciex, Concord, Ontario, Canada). Hormones were quantified in the negative ion mode, considering the recovery rates of each sample with the deuterium-labeled internal standards and generating calibration curves for each analyte by using the MultiQuant TM 3.0.1 software.

2.7. Statistical analysis

To determine the effect of "condition" and "time", mean values were tested by two-way analyses of variance (ANOVA). Condition refers here to treatment effects (comparison of stressed plants with irrigated plants). Multiple comparisons were tested with Tukey's post hoc test. All statistical differences were considered significant when $P \leq 0.05$. Both data normality (Shapiro-Wilk test) and homocedasticity of residuals (Levene's test) were previously checked as described by Zuur et al. (2009). Whenever necessary, data was transformed logarithmically to achieve normality and homocedasticity requirements to perform ANOVAs. A Pearson's correlation was performed for all parameters using a total of 102 observations. All statistical tests were performed with RStudio (RStudio Team, 2021).

2.8. Analysis of responsive elements of tocopherol biosynthetic genes

Analysis of ABA and jasmonates responsive elements (ABREs and JREs, respectively) in promoter regions of genes involved in tocopherol biosynthesis in the CAM species *Kalanchoë fedtschenkoi*, which was the species most closely related to *S. tectorum* for which genetic sequence data was publicly available (both from family *Crassulaceae*), was performed. DNA sequences were obtained from Phytozome database (https://phytozome-next.jgi.doe.gov/), where we identified the coding sequence (CDS), the 5′ untranslated region (5′UTR), including the promoter region, and the 3′ untranslated region (3′UTR, Suppl. Figs. 1–4).

3. Results

3.1. Time-course evolution of leaf water content and visual phenotype

The stress treatment imposed on *S. tectorum* plants affected their leaf water status over the weeks, with the relative water content decreasing up to 20% and the leaf hydration values decreasing from 20 to 7 g H_2O/g dry matter, which was a 65% reduction from the initial levels in the stressed group (Fig. 1). A differential dynamic in the reductions of these two parameters (RWC and H) was observed. While RWC decreased clearly from week 4 to kept constant later from week 6–10 in stressed plants, H started to differentiate between non-irrigated and irrigated plants at week 6 and then decreased progressively up to the end of the experiment, so that minimum levels were attained after 10 weeks of stress. An analysis of the visual phenotype of plants revealed that the rosette structure of the stressed plants remained intact during the experiment, although the leaves were smaller, and the outermost leaves were drying out at week 10 (Fig. 1). However, apart from this dehydration in the outer part of the rosette, which was also reflected in the monitored mid part of the rosette with the leaf fresh mass, no reductions in leaf dry mass occurred in the sampled leaves (Fig. 2).

3.2. Time-course evolution of stress-related phytohormones contents

Endogenous foliar ABA content showed a 4.2-fold increase at four weeks of stress. This sharp increase occurred specifically from two to four weeks of starting the combination of water deficit and nutrient deprivation stresses and then the levels remained constant at around 600 ng/g DW up to ten weeks of stress (Fig. 3). In contrast, foliar ABA content in irrigated plants remained constant throughout the experiment, always at values below 170 ng/g DW. Compared to ABA content, SA content in these irrigated plants was at least one order of magnitude higher ranging between 2200 and 3400 ng/g DW throughout the study. Foliar SA content decreased in stressed plants, particularly from week 6–8, reaching minimum levels below 900 ng/g DW at the end of the experiment, which represents a 60% reduction relative to controls at the end of the experiment (Fig. 3).

Moreover, the combination of water deficit with nutrient deprivation altered jasmonates metabolism, particularly from week 4 and onwards (Fig. 4). The JA precursor, OPDA decreased progressively with stress, but particularly from week 2 to week 4 of stress, during which period levels decreased from 120 to 49 ng/g DW. Later, levels decreased even further, attaining values of 15 ng/g DW at week 6 in stressed plants, remaining then that low but constant until week 10 (Fig. 4). Also, free JA showed a 66% reduction on its content after ten weeks, which dropped from 15 to 5 ng/g DW in the stressed plants, whereas JA values kept virtually constant at around 20 ng/g DW in irrigated plants (Fig. 4). Reductions in JA content were particularly evident at week 4 of stress, in which minimum values were already attained. In contrast, JA-Ile levels increased 3.6-fold after four weeks of stress, simultaneously to ABA increases and OPDA and JA reductions, to increase even further as the stress progressed, attaining maximum levels that were 5-fold higher compared to irrigated plants after ten weeks of treatments. JA-Ile ranged always at levels that were quantitatively much lower than those of JA, and the latter at lower values than those of ODPA (Fig. 4).

3.3. Linking phytohormones with photoinhibition and photoprotection

Chl loss was observed in stressed plants when compared to irrigated plants, as reflected by their total Chl content, which were 50% lower in stressed plants than in controls at week 10 (Fig. 5). This was accompanied by a reduction of 20% in the Chl *a/b* ratio at week 10 (Fig. 5).

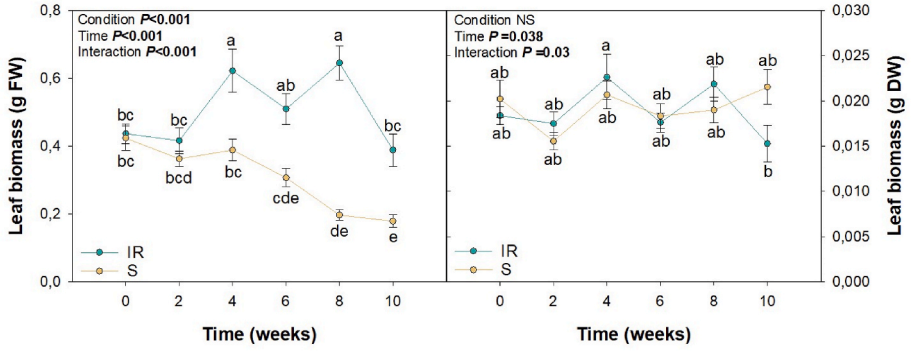

Fig. 2. Leaf mass estimation of *Sempervivum tectorum* plants during the stress acclimation process over 10 weeks. Leaf mass was expressed per gram of fresh matter (FW) and per gram of dry matter (DW). *P* values of two-way ANOVAs are shown (values above 0.05 were considered not significant [NS]). Different letters indicate significant differences between conditions and time. Data are the mean of n = 8 plants for the irrigated (IR) group and n = 9 plants for the stressed (S) group.

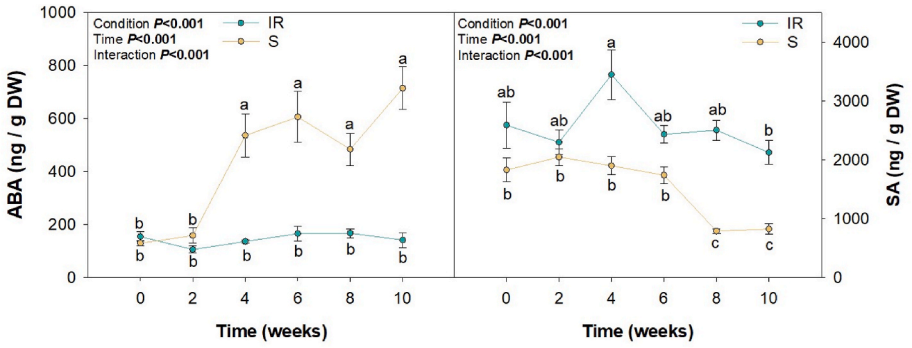

Fig. 3. Abscisic acid (ABA) and salicylic acid (SA) contents in *Sempervivum tectorum* leaves of both irrigated and non-irrigated groups through the stress acclimation process over 10 weeks. *P* values of two-way ANOVAs are shown. Different letters indicate significant differences between conditions and time. Data are the mean of n = 8 plants for the irrigated (IR) group and n = 9 plants for the stressed (S) group.

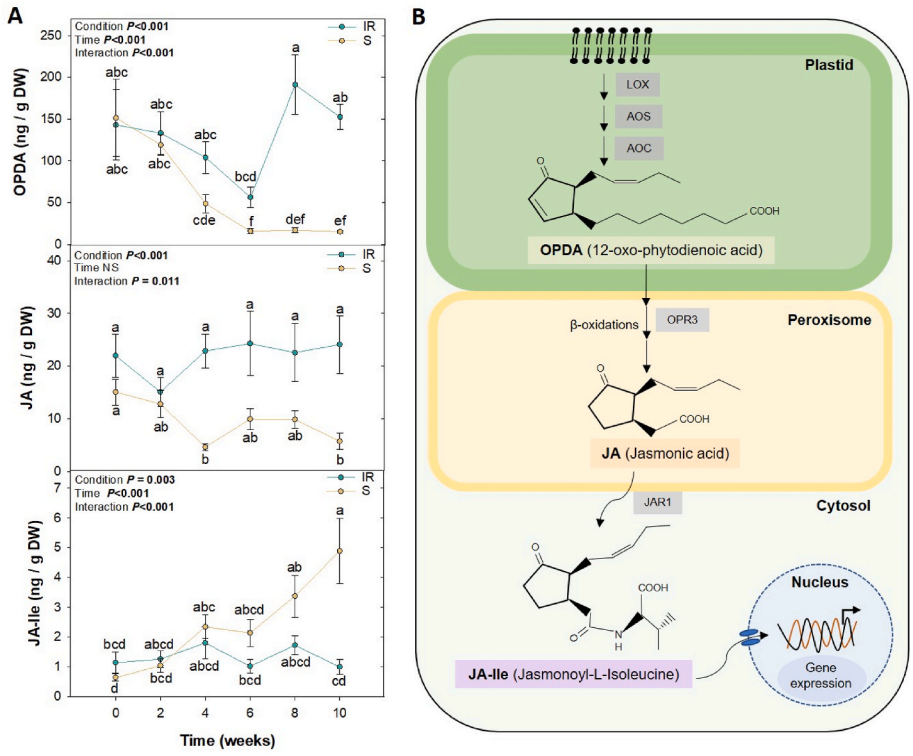

Fig. 4. Time-course evolution of jasmonate contents during the acclimation of *Sempervivum tectorum* to a combination of water deficit and nutrient deprivation. (**A**) Endogenous contents of the bioactive jasmonate, jasmonoyl-isoleucine (JA-Ile) and its precursors, 12-*oxo*-phytodienoic acid (OPDA) and jasmonic acid (JA) in leaves of *S. tectorum* during the stress acclimation process. *P* values of two-way ANOVAs are shown (values above 0.05 were considered not significant [NS]). Different letters indicate significant differences between conditions and time. Data are the mean of n = 8 plants for the irrigated (IR) group and n = 9 plants for the stressed (S) group. (**B**) Diagram showing the jasmonates biosynthetic pathway. AOC: allene oxide cyclase; AOS: allene oxide synthase; JAR1: jasmonic acid reductase; LOX: lipoxygenase; OPR3: 12-*oxo*-phytodienoic acid reductase.

However, stressed plants did not show any symptom of neither photoinhibition nor photo-oxidative damage, as indicated by F_v/F_m values above 0.75 and constant MDA content throughout the experiment (Fig. 5). Although the effect of treatment was significant ($P = 0.038$) for MDA, its content ranged around 20 nmol/g DW throughout the experiment in both treatments and no significant differences were found at any specific time point of measurement between non-irrigated and irrigated plants.

Despite being the major vitamin E form in *S. tectorum*, α-tocopherol content did not vary significantly throughout the study between treatments with values ranging between 220 and 300 μg/g DW (Fig. 6). In contrast, γ-tocopherol (the immediate precursor of α-tocopherol) was present at lower, although still quite remarkable amounts, with a content 65% lower to that observed for α-tocopherol. Notably, γ-tocopherol content decreased progressively in the stressed plants and it was significantly lower in the non-irrigated than in the irrigated group at week 8. No differences were observed in irrigated plants over time, but a significant reduction was found for the stressed plants at weeks 8 and 10 compared to the initial values (Fig. 6).

When expressed on a Chl basis, α-tocopherol content showed more remarkable significant differences between treatments, but those previously observed for γ-tocopherol almost disappeared (Fig. 6). Although treatment had a significant effect ($P = 0.021$) in the γ-tocopherol content expressed on a Chl basis, no significant effect of the interaction between condition and time was found and post hoc treatment analysis did not reveal any significant difference at specific time points between controls and stressed plants (Fig. 6). This indicates that Chl loss occurred quite in parallel with γ-tocopherol reduction when expressed on a dry matter basis (Figs. 5 and 6). A completely different result due to Chl loss was observed for α-tocopherol. An increase of 44% in α-tocopherol per unit of Chl was observed at four weeks of water deficit, being the levels significantly different from this week onwards relative to controls. Besides, α-tocopherol levels per unit of Chl in plants exposed to a combination of water deficit and nutrient deprivation attained maximum levels of 405 mmol/mol at week 10, values that were 2.3-fold higher than those of irrigated plants (Fig. 6). This time-course evolution

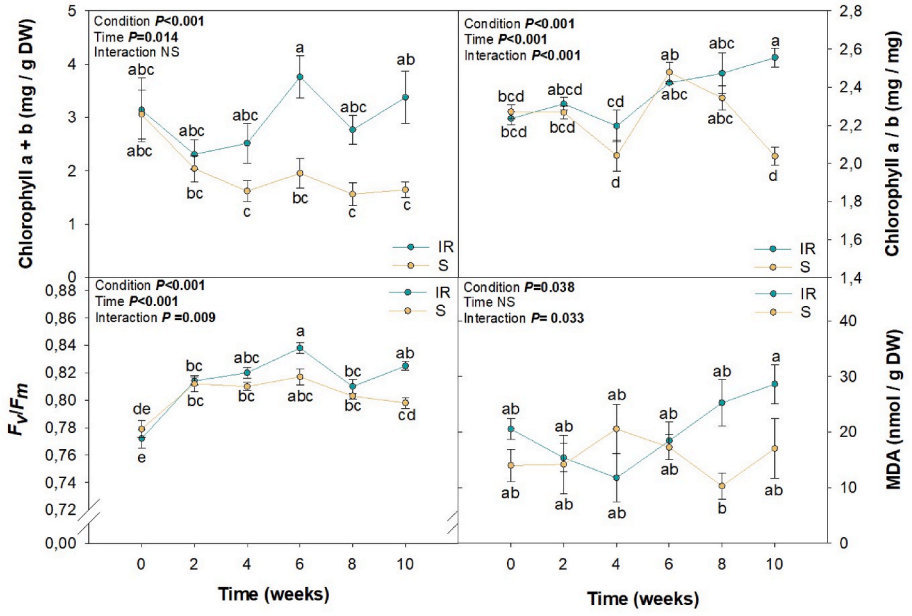

Fig. 5. Time-course evolution of total chlorophyll contents (chlorophyll *a*+b), chlorophyll *a*/b, the maximum efficiency of photosystem II (F_v/F_m) and the lipid peroxidation product, malondialdehyde (MDA) in *Sempervivum tectorum* leaves of both irrigated (IR) and stressed (S) plants over 10 weeks. *P* values of two-way ANOVAs are shown (values above 0.05 were considered not significant [NS]). Different letters indicate significant differences between conditions and time. Data are the mean of n = 8 plants for the IR group and n = 9 plants for the S group.

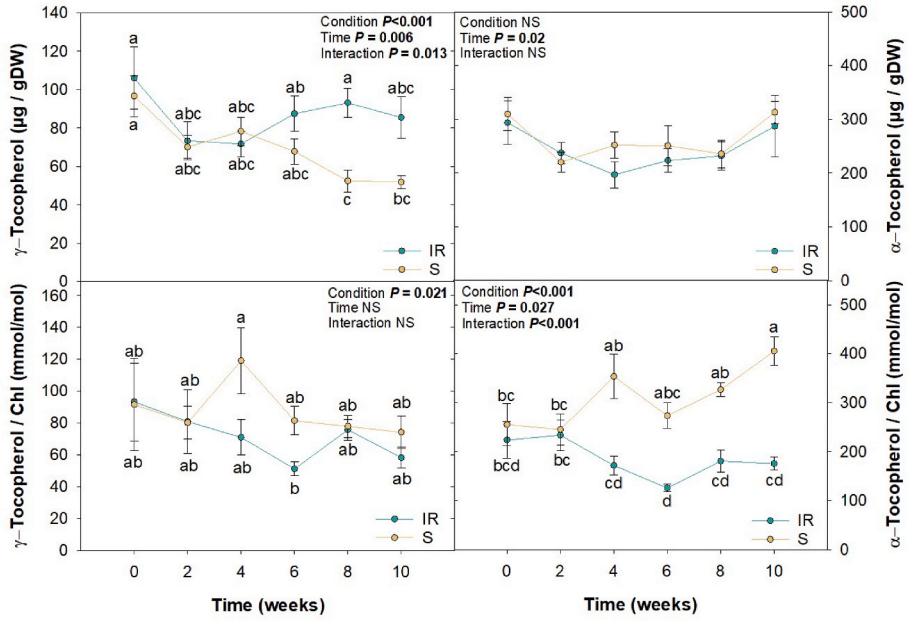

Fig. 6. Foliar vitamin E (including both α and γ-tocopherol) contents in *Sempervivum tectorum* leaves of both irrigated and non-irrigated groups through the stress acclimation process over 10 weeks. Data was expressed in both on a dry mass (DW) and on a chlorophyll *a*+b (Chl) basis. *P* values of two-way ANOVAs are shown (values above 0.05 were considered not significant [NS]). Different letters indicate significant differences between conditions and time. Data are the mean of n = 8 plants for the irrigated (IR) group and n = 9 plants for the stressed (S) group.

paralleled that of ABA (Fig. 2) and JA-Ile (Fig. 3), which was confirmed by correlative studies. Pearson correlation showed that among the stress-related phytohormones measured, the highest positive correlation for α-tocopherol levels per unit of Chl was observed with JA-Ile followed by ABA (Fig. 7). This ratio also highly negatively correlated, indeed even slightly better, with leaf hydration. A remarkable negative correlation for α-tocopherol levels per unit of Chl was also observed with leaf fresh mass, whereas the correlation was positive with γ-tocopherol levels per unit of Chl (Fig. 7). In contrast, remarkable correlations (with r and *P* values above 0.4 and below 0.001, respectively, *sensu* Zuur et al., 2009) were not observed for α-tocopherol content expressed per unit of dry mass with any other studied parameter, except with its precursor γ-tocopherol and with Chl content. However, a moderate correlation was also observed with ABA ($r = 0.30$, $P < 0.01$, Fig. 7). Analysis of ABA and jasmonates responsive elements in promoter regions of genes involved in tocopherol biosynthesis in the CAM species *Kalanchoë fedtschenkoi*, which was the species most closely related to *S. tectorum* for which genetic sequence data was publicly available, revealed that both ABA and jasmonates-related cis-regulatory elements are present in genes encoding biosynthetic enzymes of tocopherol (Fig. 8). More specifically, we found JREs in the promoter of the gene encoding for *p*-hydroxyphenolpyruvate dioxygenase (HPPD), which is essential for providing homogentisate building blocks for the chromanol ring of all tocopherols, and ABREs in the promoter of the gene encoding for γ-tocopherol methyl transferase (VTE4), which catalyses the last step of α-tocopherol biosynthesis from γ-tocopherol (Fig. 8).

4. Discussion

An early plant response to drought events clearly defines the drought acclimation ability of a plant species and may condition its survival during later stages of stress. CAM plants have convergently evolved

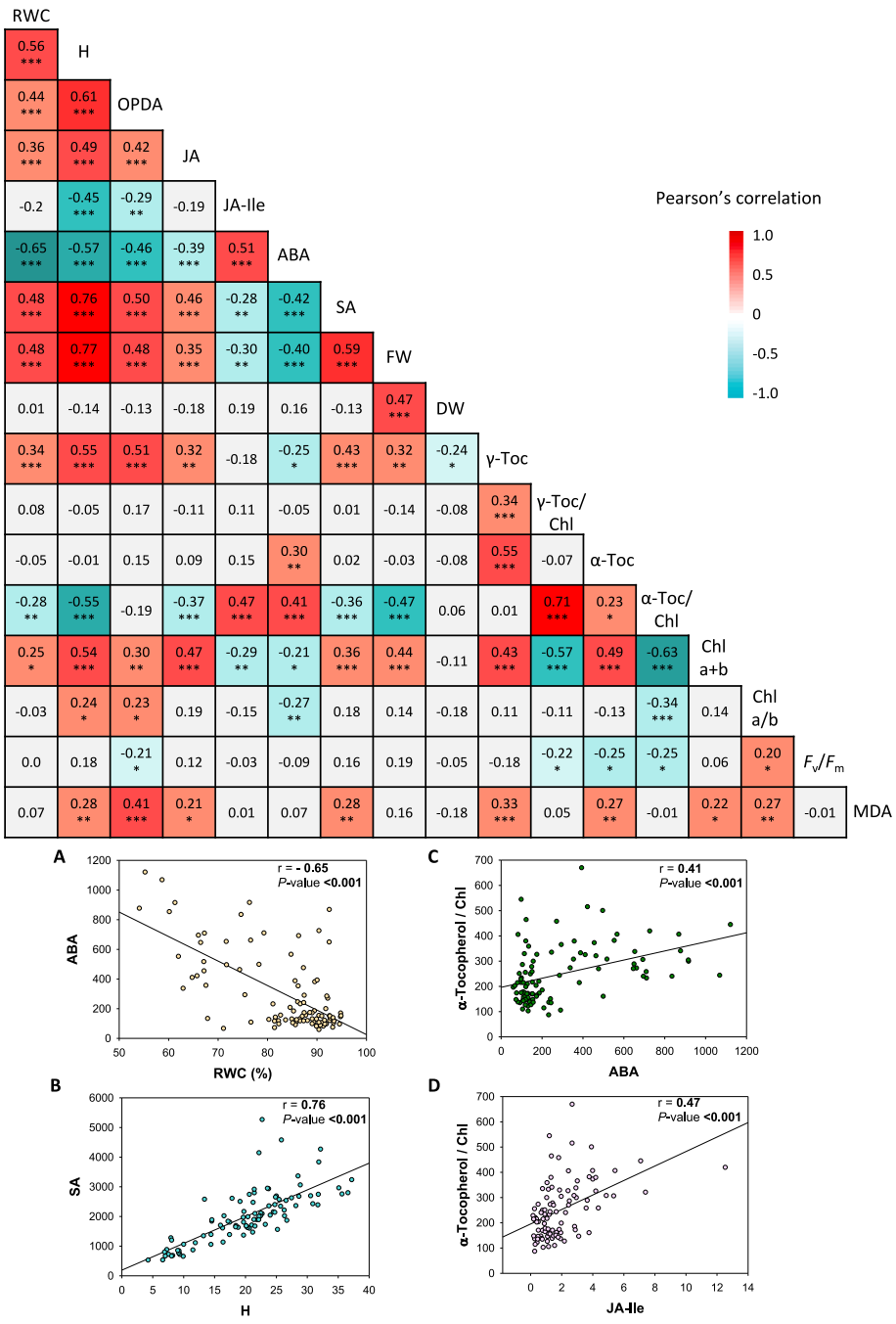

Fig. 7. Pearson correlation matrix showing r coefficient values between the different studied parameters. One, two and three asterisks indicate *P* values < 0.05, <0.01 and < 0.001, respectively. The color gradient indicates ranges of correlation from red (positive) to blue (negative) and only significant correlations (*p* value < 0.05) are shown in color. Graphs below show the most biologically significant correlations including: (A) Negative correlation between abscisic acid (ABA) and relative water content (RWC), (B) Positive correlation between salicylic acid (SA) and leaf hydration (H), (C) Positive correlation between ABA and α-tocopherol per unit of chlorophyll (α-Toc/Chl), and (D) Positive correlation between jasmonoyl-isoleucine (JA-Ile) and the α-Toc/Chl ratio with a total of N = 102 observations. Chl, total chlorophyll; DW, dry matter; *Fv/Fm*, maximum photochemical efficiency of photosystem II; FW, fresh weight; JA, Jasmonic acid; MDA, malondialdehyde; OPDA, 12-*oxo*-phytodienoic acid; γ-Toc, γ-Tocopherol. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

many times among distant lineages, through generations of acclimation to drought stress in their arid natural habitats, which may face water deficit with combined nutrient deprivation in poor nutrient soils and has resulted in genetic changes that have provided their current metabolic adaptation to several abiotic stress (Heyduk et al., 2019). Thus, it is known that CAM plants can quickly respond to water deficit and withstand long dry spells, but their drought response acclimation mechanisms are still being disentangled, particularly when water deficit occurs in combination with nutrient deficiency. Here, we showed that the contents of endogenous stress-related phytohormones follow a very specific time-course evolution in *S. tectorum*, with simultaneous increases in ABA and JA-Ile at four weeks of combined water deficit and nutrient deprivation stress, in which the content of OPDA and JA decrease while those of JA-Ile increase, thus suggesting a specific activation of JASMONATE RESISTANT 1 (JAR1), the enzyme responsible for conversion from JA to JA-Ile, under stress. These findings support our initial hypothesis that not only ABA but also JA-Ile may be involved in the plant response to a combination of water deficit and nutrient deprivation in *S. tectorum*. However, our hypothesis that ABA and JA-Ile simultaneously increase with vitamin E accumulation in leaves resulted to be untrue, at least in part. ABA, but not jasmonates (including JA-Ile), positively correlated with α-tocopherol, but intriguingly both ABA and JA-Ile contents correlated with the accumulation of α-tocopherol per unit of Chl, although not with the content of γ-tocopherol per unit of Chl.

The water stress response was characterized by an increase in ABA concentrations at four weeks of stress to keep later constant at high levels throughout the experiment. Although ABA is the phytohormone promoting stomatal closure in plants (Suhita et al., 2004), measurements were always performed at midday on clear sunny days in which CAM plants show severe stomatal closure, so this peak seemed more

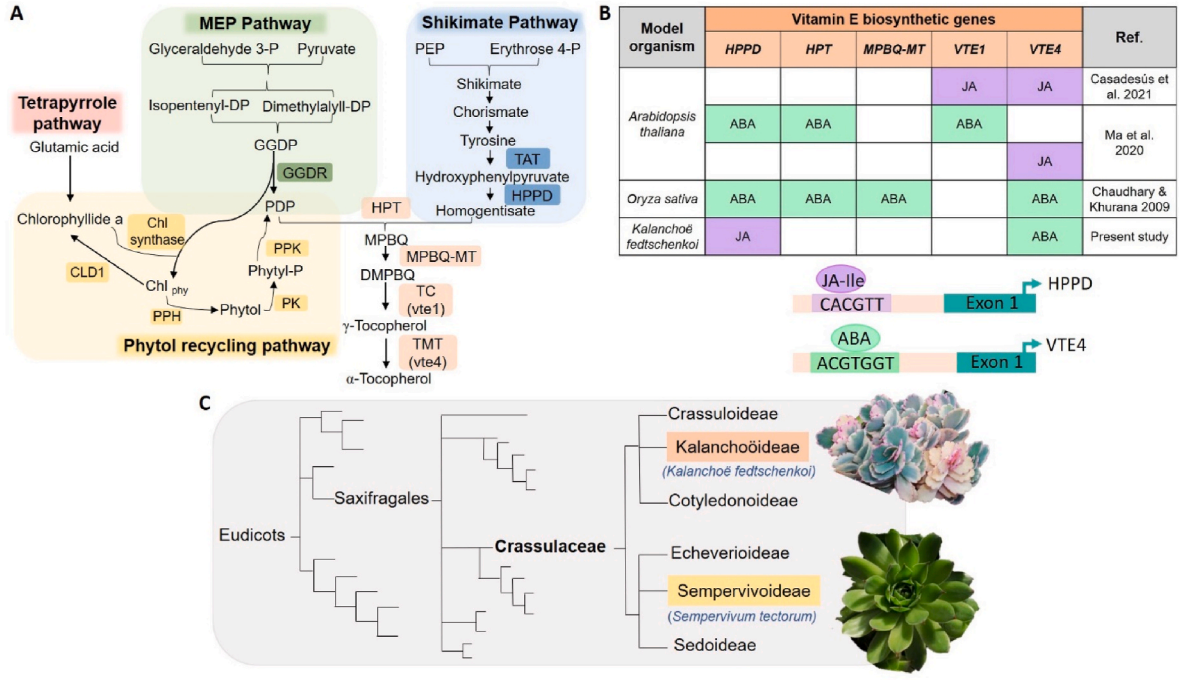

Fig. 8. Tocopherol biosynthesis regulation in a CAM plant. (**A**) Scheme of tocopherol biosynthetic pathway in plants. (**B**) Table showing the vitamin E biosynthetic genes in which regulatory sequences responsive elements to abscisic acid (ABA) and/or jasmonates (JA) have been found in either the model organism *Arabidopsis thaliana*, *Oryza sativa* or the CAM species *Kalanchoë fedtschenkoi*. Sequences with JRE or ABRE in promoters of the *HPPD* and *VTE4* genes found in the CAM plant *K. fedtschenkoi* are shown below (see also Suppl. File 1). (**C**) Evolutionary classification of *Crassulaceae* family, in which the *Sempervivoidae* and *Kalanchoöideae* subfamilies are found, to which *Sempervivum tectorum* and *Kalanchoë fedtschenkoi* belong, respectively. CLD1 chlorophyll dephytylase, DMPBQ dimethylphytylbenzoquinol, GGDP geranylgeranyl diphosphate, GGDR gernaylgernayl diphosphate reductase, HPPD hydroxyphenylpyruvate dioxygenase, HPT homogentisate phytyl transferase, MEP methylerythriol phosphate, MPBQ methylphytylbenzoquinol, MPBQ-MT methylphytylbenzoquinol methyltransferase, PEP phosphoenolpyruvate, PDP phytyl diphosphate, PK phytyl kinase, PPH pheophytin pheophorbide hydrolase, PPK phytyl phosphate kinase, TAT tyrosine aminotransferase, TC tocopherol cyclase, TMT tocopherol methyltransferase.

related to preserve plant water status during these first weeks of stress by modulating other physiological processes related to water relations. Previous evidence shows that ABA is involved in the activation of non-stomatal, morphological and biochemical mechanisms modulating water-use efficiency, such as changes in leaf size, osmotic adjustment, and even root development, thus favouring acclimation to nutrient poor soils (Negin and Moshelion, 2016). Intriguingly, among the various parameters studied, ABA content not only strongly negatively correlated with the relative water content and leaf hydration, but also with OPDA and SA contents and with leaf fresh mass, and it positively correlated with α-tocopherol per unit of Chl. In turn, SA content strongly positively correlated with leaf hydration, relative water content and contents of both OPDA and JA; and while relative water content did not decrease further after four week of stress, leaf hydration continued slightly decreasing throughout the experiment. These results suggest that ABA may antagonistically modulate together with SA some aspects related to water deficit-induced morphological changes. Most notably, visual symptoms of combined water deficit and nutrient deprivation stress and the reductions in leaf hydration suggest that leaves were simply smaller and showed desiccation at 10 weeks of stress. The fact that relative leaf water content and leaf dry mass kept constant from week 6–10 onwards suggests that these smaller leaves (very likely with thicker cell walls and smaller vacuoles) are tolerant to desiccation. Indeed, this is consistent with previous studies evaluating the tolerance of this plant species to high temperatures, showing a tremendous degree of tolerance (Larcher and Wagner, 1983; Larcher et al., 1989, 2010).

Biosynthesis of the bioactive jasmonate form, JA-Ile, increased in the stressed plants at week 4, with values reaching their maximum levels at week 10. Despite its strong association with the biotic stress response (Heyer et al., 2018), the role of jasmonates in abiotic stress tolerance, including drought stress, has also been previously described (Sánchez-Romera et al., 2014; Cotado et al., 2018), although not in CAM plants. Here, we showed for the first time a JA-Ile accumulation in a CAM plant facing a combination of water deficit and nutrient deprivation, while OPDA and JA decreased, suggesting that JA-Ile is the bioactive molecule of the jasmonate signaling pathway involved in the response in *S. tectorum* to this stress combination, which has been previously described for other species (more specifically in the model plant *A. thaliana*) in the case of water deficit stress or nutrient deprivation (such as P starvation) applied alone, but neither with both stresses combined nor in CAM plants (Fonseca et al., 2009; Wasternack and Hause, 2013; Khan et al., 2015). Regulation of vitamin E synthesis by ABA and jasmonates has been proposed in several studies for other species (Sandorf and Holländer-Czytko, 2002; El Kayal et al., 2006; Siles et al., 2018), and interestingly both ABA and JA-Ile contents correlated with the accumulation of α-tocopherol per unit of Chl in *S. tectorum*, although not with the content of γ-tocopherol per unit of Chl. Several genes from the vitamin E biosynthetic pathway have been examined in their promoter regions to detect responsive elements to specific hormones, but mostly in model organisms (Fig. 8A). For instance, ABREs were found in *HPPD*, *MPBQ-MT* and *TMT* (*VTE4*) genes in the C3 plant *Oryza sativa*, and a motif IIb was also found in *OsHPT* (Chaudhary and Khurana, 2009), suggesting a specific regulation of vitamin E by ABA. Likewise, jasmonates responsive elements were found in *VTE1* and *VTE4* in *Arabidopsis thaliana*, encoding genes for the tocopherol cyclase and tocopherol methyl transferase, respectively (Ma et al., 2020; Casadesús et al., 2021). Although much less research about responsive elements of vitamin E-related biosynthesis genes has been performed in non-model organisms, such as CAM species, advances on the sequencing of whole genomes of CAM plants have recently been made, including both monocots, with the orchid *Phalaenopsis equestris* (Cai et al., 2015) and the pineapple *Ananas cosmosus* (Ming et al., 2015), and one eudicot of

the *Crassulaceae* family, *Kalanchoë fedtschenkoi* (Yang et al., 2017). Since *K. fedtschenkoi* belongs to the same family as *S. tectorum* and it is the phylogenetically closest obligate CAM species with available genome sequences of vitamin E biosynthetic genes, we explored the possibility of finding JREs and ABREs in this species (Fig. 8C). Here, we identified a JRE in the promoter region of *HPPD* and an ABRE in the promoter region of *VTE4* in the CAM plant *K. fedtschenkoi* (Suppl. File 1), thus suggesting that both jasmonates and ABA might synergistically enhance α-tocopherol biosynthesis, which is consistent with biochemical data. Thus, our results, although limited by its correlative nature, suggest a photoprotective activation role for these phytohormones in which ABA and JA-Ile may cooperatively promote vitamin E biosynthesis in *S. tectorum*. Most importantly, results suggest that both group of hormones complement each other to specifically increase the content of α-tocopherol per unit of Chl, a ratio that reflects the capacity of photoprotection of this antioxidant molecule per number of photons absorbed (*sensu* Kyparissis et al., 1995; see also Munné-Bosch and Alegre, 2000). α-Tocopherol plays an essential role in chloroplasts in the protection of the photosynthetic apparatus preventing the propagation of lipid peroxidation, both by scavenging lipid peroxyl radicals and eliminating reactive oxygen species, such as singlet oxygen and hydroxyl radicals (Havaux et al., 2005; Mesa and Munné-Bosch, 2023), as well as in the protection of PSII from photoinhibitory damage by preventing singlet oxygen-related net loss of the D1 protein (Kumar et al., 2021). Thus, an increase in α-tocopherol per unit of Chl in the stressed *S. tectorum* plants may be very helpful to reduce photo-oxidative stress in chloroplasts working at high oxygen tensions and excess light. In this regard, it is also very important to consider the biological significance of Chl loss throughout the study, which up to week 8 was accompanied by a constant Chl *a*/*b* ratio, thus suggesting a reduction in the light harvesting antennae that may strongly decrease the number of excess photons received during the day. Since ABA and jasmonates have also been implicated in the regulation of Chl degradation and leaf senescence (Tsuchiya et al., 1999; Zhao et al., 2016), it is noteworthy that a part of the photoprotective response observed here was simply related to an induction of leaf senescence, a process that may occur slowly and progressively to provide sufficient time for nutrient remobilization, a process that results to be essential for acclimation to a combination of water deficit and nutrient deprivation (Munné-Bosch and Alegre, 2004; Zhao et al., 2016). Interestingly, at the end of the experiment the Chl *a*/*b* ratio decreased significantly together with total Chl loss, which may be associated with a reduction in reaction centers, as only Chl *a* is located in them, whereas both Chl *a* and Chl *b* occur in light harvesting complexes (Croce and van Amerongen, 2011). Therefore, it is very likely that sampled leaves in the mid rosette will suffer from photoinhibition and advanced symptoms of leaf senescence under more severe stress. Future studies are warranted to unravel the limits of stress tolerance in this plant species and what are the underlying mechanisms.

5. Conclusions

It is concluded that *S. tectorum* can withstand a combination of water deficit and nutrient deprivation for almost three months without suffering signs of photoinhibition, avoiding photo-oxidative stress and photo-oxidative damage in the central part of its rosette. Also, results show that this species is endowed with a physiological mechanism in which not only ABA but also the bioactive jasmonate form, JA-Ile, may induce photoprotection, particularly by increasing the α-tocopherol per unit of Chl ratio, thus displaying an early effective stress acclimation strategy. The exact signaling pathways underlying this response remain, however, elusive, but results shown here suggest that both ABA and jasmonates may interact in the observed response and add to our current knowledge that both groups of phytohormones interact in the modulation of plant responses to abiotic stress, also in CAM plants.

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CRediT authorship contribution statement

Sabina Villadangos: Formal analysis, Conceptualization, Data curation, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. **Sergi Munné-Bosch:** Conceptualization, Funding acquisition, Investigation, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jplph.2023.154040.

References

Abram, V., Donko, M., 1999. Tentative identification of polyphenols in *Sempervivum tectorum* and assessment of the antimicrobial activity of *Sempervivum* L. J. Agric. Food Chem. 47, 485–489.

Amaral, J.S., Casal, S., Torres, D., Seabra, R.M., Oliveira, B.P.P., 2005. Simultaneous determination of tocopherols and tocotrienols in hazelnuts by a normal phase liquid chromatographic method. Anal. Sci. 21, 1545–1548.

Bosch, M., Wright, L.P., Gershenzon, J., Wasternack, C., Hause, B., Schaller, A., Stintzi, A., 2014. Jasmonic acid and its precursor 12-oxophytodienoic acid control different aspects of constitutive and induced herbivore defenses in tomato. Plant Physiol. 166, 396–410.

Cai, J., Liu, X., Vanneste, K., Proost, S., Tsai, W.C., Liu, K.W., Chen, L.J., He, Y., Xu, Q., Bian, C., Zheng, Z., Sun, F., Liu, W., Hsiao, Y.Y., Pan, Z.J., Hsu, C.C., Yang, Y.P., Hsu, Y.C., Chuang, Y.C., Dievart, A., Dufayard, J.F., Xu, X., Wang, J.Y., Wang, J., Xiao, X.J., Zhao, X.M., Du, R., Zhang, G.Q., Wang, M., Su, Y.Y., Xie, G.C., Liu, G.H., Li, L.Q., Huang, L.Q., Luo, Y.B., Chen, H.H., Van de Peer, Y., Liu, Z.J., 2015. The genome sequence of the orchid *Phalaenopsis equestris*. Nat. Genet. 47, 65–72.

Casadesús, A., Bouchikh, R., Pérez-Llorca, M., Munné-Bosch, S., 2021. Linking jasmonates with vitamin E accumulation in plants: a case study in the Mediterranean shrub *Cistus albidus* L. Planta 253, 36.

Cela, J., Arrom, L., Munné-Bosch, S., 2009. Diurnal changes in photosystem II photochemistry, photoprotective compounds and stress-related phytohormones in the CAM plant, *Aptenia cordifolia*. Plant Sci. 177, 404–410.

Chaudhary, N., Khurana, P., 2009. Vitamin E biosynthesis genes in rice: molecular characterization, expression profiling and comparative phylogenetic analysis. Plant Sci. 177, 479–491.

Cotado, A., Müller, M., Morales, M., Munné-Bosch, S., 2018. Linking jasmonates with pigment accumulation and photoprotection in a high-mountain endemic plant, *Saxifraga longifolia*. Environ. Exp. Bot. 154, 56–65.

Croce, R., van Amerongen, H., 2011. Light-harvesting and structural organization of Photosystem II: from individual complexes to thylakoid membrane. J. Photochem. Photobiol., B 104, 142–153.

Davies, P.J., 2010. Plant Hormones: Biosynthesis, Signal Transduction. Action! Springer, Dordrecht, The Netherlands.

De Ollas, C., Arbona, V., Gómez-Cadenas, A., Dodd, I.C., 2018. Attenuated accumulation of jasmonates modifies stomatal responses to water deficit. J. Exp. Bot. 69, 2103–2116.

El Kayal, W., Keller, G., Debayles, C., Kumar, R., Weier, D., Teulieres, C., Marque, C., 2006. Regulation of tocopherol biosynthesis through transcriptional control of

tocopherol cyclase during cold hardening in *Eucalyptus gunnii*. Physiol. Plant. 126, 212–223.
Fleta-Soriano, E., Pintó-Marijuan, M., Munné-Bosch, S., 2015. Evidence of drought stress memory in the facultative CAM, *Aptenia cordifolia*: possible role of phytohormones. PLoS One 10, e0135391.
Fonseca, S., Chini, A., Hamberg, M., Adie, B., Porzel, A., Kramell, R., Miersch, O., Wasternack, K., Solano, R., 2009. (+)-7-*iso*-Jasmonoyl-L-isoleucine is the endogenous bioactive jasmonate. Nat. Chem. Biol. 5, 344–350.
Forseth, I.N., 2010. The ecology of photosynthetic pathways. Nat. Educ. Knowl. 3, 4.
Glušac, J., Morina, F., Veljović-Jovanović, S., Boroja, M., Kukavica, B., 2013. Changes in the antioxidative metabolism induced by drought and Cd excess in the leaves of houseleek (*Sempervivum tectorum* L.). Fresenius Environ. Bull. 22, 1770–1776.
Havaux, M., Eymery, F., Porfirova, S., Rey, P., Dörmann, P., 2005. Vitamin E protects against photoinhibition and photooxidative stress in *Arabidopsis thaliana*. Plant Cell 17, 3451–3469.
Hewedy, O.A., Elsheery, N.I., Karkour, A.M., Elhamouly, N., Arafa, R.A., Mahmoud, G.A., Dawood, M.F.A., Hussein, W.E., Mansour, A., Amin, D.H., Allakhverdiev, S.I., Zivcak, M., Brestic, M., 2023. Jasmonic acid regulates plant development and orchestrates stress response during tough times. Environ. Exp. Bot. 208, 105260.
Heyduk, K., Moreno-Villena, J.J., Gilman, I.S., Christin, P.A., Edwards, E.J., 2019. The genetics of convergent evolution: insights from plant photosynthesis. Nat. Rev. Genet. 20, 485–493.
Heyer, M., Reichelt, M., Mithöfer, A., 2018. A holistic approach to analyze systemic jasmonate accumulation in individual leaves of *Arabidopsis* rosettes upon wounding. Front. Plant Sci. 9, 1569.
Hodges, D.M., DeLong, J.M., Forney, C.F., Prange, R.K., 1999. Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. Planta 207, 604–611.
Ilyas, M., Nisar, M., Khan, N., Hazrat, A., Khan, A.M., Hayat, H., Fahad, S., Khan, A., Ullah, A., 2021. Drought tolerance strategies in plants: a mechanistic approach. J. Plant Growth Regul. 40, 926–944.
Kazan, K., 2015. Diverse roles of jasmonates and ethylene in abiotic stress tolerance. Trends Plant Sci. 20, 219–229.
Khan, G.A., Vogiatzaki, E., Glauser, G., Poirier, Y., 2015. Phosphate deficiency induces the jasmonate pathway and enhances resistance to insect herbivory. Plant Physiol. 171, 632–644.
Khan, M.I.R., Fatma, M., Per, T.S., Anjum, N.A., Khan, N.A., 2015. Salicylic acid-induced abiotic stress tolerance and underlying mechanisms in plants. Front. Plant Sci. 6, 462.
Klein, J.T., Kaderit, J.W., 2015. Phylogeny, biogeography, and evolution of edaphic association in the European oreophytes *Sempervivum* and *Jovibarba* (Crassulaceae). Int. J. Plant Sci. 176, 44–71.
Kondo, A., Kaikawa, J., Funaguma, T., Ueno, O., 2004. Clumping and dispersal of chloroplasts in succulent plants. Planta 219, 500–506.
Kumar, A., Prasad, A., Sedlarova, M., Kale, R., Frankel, L.K., Sallans, L., Bricker, T.M., Prospisil, P., 2021. Tocopherol controls D1 amino acid oxidation by oxygen radicals in Photosystem II. Proc. Natl. Acad. Sci. USA 118, e2019246118.
Kyparissis, A., Petropoulou, Y., Manetas, Y., 1995. Summer survival of leaves in a soft-leaved shrub (*Phlomis fructicosa* L., Labiatae) under Mediterranean field conditions: avoidance of photoinhibitory damage through decreased chlorophyll contents. J. Exp. Bot. 46, 1825±1831.
Larcher, W., Wagner, J., 1983. Ökologischer Zeigerwert und physiologische Konstitution von *Sempervivum montanum*. Verh. Ges. f. Ökol. 11, 253–264.
Larcher, W., Holzner, M., Pichler, J., 1989. Temperaturresistenz inneralpiner Steppengräser. Flora 183, 115–131.
Larcher, W., Kainmüller, C., Wagner, J., 2010. Survival types of high mountain plants under extreme temperatures. Flora 205, 3–18.
Lichtenthaler, H., Wellburn, A., 1983. Determinations of total carotenoids and chlorophylls *a* and *b* of leaf extracts in different solvents. Biochem. Soc. Trans. 11, 591–592.
Ma, J., Qiu, D., Pang, Y., Gao, H., Wang, X., 2020. Diverse roles of tocopherols in response to abiotic and biotic stresses and strategies for genetic biofortification in plants. Mol. Breed. 40, 18.
Mesa, T., Munné-Bosch, S., 2023. α-Tocopherol in chloroplasts: Nothing more than an antioxidant? Curr. Opin. Plant. Biol. 74, 102400.
Ming, R., VanBuren, R., Wai, C.M., Tang, H., Schatz, M.C., Bowers, J.E., Lyons, E., Wang, M.L., Chen, J., Briggers, E., Zhang, J., Huang, L., Zhang, L., Miao, W., Zhang, J., Ye, Z., Miao, C., Lin, Z., Wang, H., Zhou, H., Yim, W.C., Priest, H.D., Zheng, C., Woodhouse, M., Edger, P.P., Guyot, R., Guo, H.B., Guo, H., Zheng, G., Singh, R., Sharma, A., Min, X., Zheng, Y., Lee, H., Gurtowski, J., Sedlazeck, F.J., Harkess, A., McKain, M.R., Liao, Z., Fang, J., Liu, J., Zhang, X., Zhang, Q., Hu, W., Qin, Y., Wang, K., Chen, L.Y., Shirley, N., Lin, Y.R., Liu, L.Y., Hernandez, A.G., Wright, C.L., Bulone, V., Tuskan, G.A., Heath, K., Zee, F., Moore, P.H., Sunkar, R., Leebens-Mack, J.H., Mockler, T., Bennetzen, J.L., Freeling, M., Sankoff, D., Paterson, A.H., Zhu, X., Yang, X., Smith, J.A.C., Cushman, J.C., Paull, R.E., Yu, Q., 2015. The pineapple genome and the evolution of CAM photosynthesis. Nat. Genet. 47, 1435–1442.
Morales, M., Garcia, Q.S., Munné-Bosch, S., 2015. Ecophysiological response to seasonal variations in water availability in the arborescent, endemic plant *Vellozia gigantea*. Tree Physiol. 35, 253–265.
Munemasa, S., Oda, K., Watanabe-Sugimoto, M., Nakamura, Y., Shimoishi, Y., Murata, Y., 2007. The *coronatine-insensitive1* mutation reveals the hormonal signaling interaction between abscisic acid and methyl jasmonate in *Arabidopsis* guard cells. Specific impairment of ion channel activation and second messenger production. Plant Physiol. 143, 1398–1407.
Munné-Bosch, S., Alegre, L., 2000. Changes in carotenoids, tocopherols and diterpenes during drought and recovery, and the biological significance of chlorophyll loss in *Rosmarinus officinalis* plants. Planta 210, 925–931.
Munné-Bosch, S., Alegre, L., 2004. Die and let live: leaf senescence contributes to plant survival under drought stress. Funct. Plant Biol. 31, 203–216.
Muñoz, P., Munné-Bosch, S., 2019. Vitamin E in plants: biosynthesis, transport, and function. Trends Plant Sci. 24, 1040–1051.
Negin, B., Moshelion, M., 2016. The evolution of the role of ABA in the regulation of water-use efficiency: from biochemical mechanisms to stomatal conductance. Plant Sci. 251, 82–89.
Parnell, J., Favarger, C., 1993. In: Tutin, T.G., Burges, N.A., Chater, A.O., Edmondson, J. R., Heywood, V.H., Moore, D.M., Valentine, D.H., Walters, S.M., Europaea, Webb D. A. Flora (Eds.), *Sempervivum* L., Jovibarba Opiz. Cambridge University Press, Cambridge, pp. 425–428.
Praeger, R.L., 1932. An Account of the *Sempervivum* Group. Royal Horticultural Society, London.
RStudio Team, 2021. RStudio. integrated development environment for R. RStudio, PBC, Boston, MA, USA.
Sánchez-Romera, B., Ruiz-Lozano, J.M., Li, G., Luu, D.T., Martínez-Ballesta, M.D., Carvajal, M., Zamarreño, A.M., García-Mina, J.M., Maurel, C., Aroca, R., 2014. Enhancement of root hydraulic conductivity by methyl jasmonate and the role of calcium and abscisic acid in the process. Plant Cell Environ. 37, 995–1008.
Sandorf, I., Holländer-Czytko, H., 2002. Jasmonate is involved in the induction of tyrosine aminotransferase and tocopherol biosynthesis in *Arabidopsis thaliana*. Planta 216, 173–179.
Savchenko, T., Kolla, V.A., Wang, C.Q., Nasafi, Z., Hicks, D.R., Phadungchob, B., Chehab, W.E., Brandizzi, F., Froehlich, J., Dehesh, K., 2014. Functional convergence of oxylipin and abscisic acid pathways controls stomatal closure in response to drought. Plant Physiol. 164, 115–160.
Siles, L., Alegre, L., González-Solís, A., Cahoon, E.B., Munné-Bosch, S., 2018. Transcriptional regulation of vitamin E biosynthesis during germination of dwarf fan palm seeds. Plant Cell Physiol. 59, 2490–2501.
Smith, M.C., 1981. *Sempervivum* (Crassulaceae) in Spain and the Pyrenees. Lagascalia 10, 1–23.
Suhita, D., Raghavendra, A.S., Kwak, J.M., Vavasseur, A., 2004. Cytoplasmic alkalization precedes reactive oxygen species production during methyl jasmonate-and abscisic acid-induced stomatal closure. Plant Physiol. 134, 1536–1545.
Takahashi, S., Badger, M.R., 2011. Photoprotection in plants: a new light on photosystem II damage. Trends Plant Sci. 16, 53–60.
Taybi, T., Cushman, J.C., Borland, A.M., 2002. Environmental, hormonal and circadian regulation of crassulacean acid metabolism expression. Funct. Plant Biol. 29, 669–678.
Thompson, A.J., Andrews, J., Mulholland, B.J., McKee, J.M.T., Hilton, H.W., Horridge, J. S., Farquhar, G.D., Smeeton, R.C., Smillie, I.R.A., Black, C.R., Taylor, I.B., 2007. Overproduction of abscisic acid in tomato increases transpiration efficiency and root hydraulic conductivity and influences leaf expansion. Plant Physiol. 143, 1905–1917.
Tsuchiya, T., Ohta, H., Okawa, K., Iwamatsu, A., Shimada, H., Masuda, T., Takamiya, K., 1999. Cloning of chlorophyllase, the key enzyme in chlorophyll degradation: finding of a lipase motif and the induction by methyl jasmonate. Proc. Natl. Acad. Sci. USA 96, 15362–15367.
Wakamatsu, A., Mori, I.C., Matsuura, T., Taniwaki, Y., Ishii, R., Yoshida, R., 2021. Possible roles for phytohormones in controlling the stomatal behavior of *Mesembryanthemum crystallinum* during the salt-induced transition from C3 to crassulacean acid metabolism. J. Plant Physiol. 262, 153448.
Wasternack, C., Hause, B., 2013. Jasmonates: biosynthesis, perception, signal transduction and action in plant stress response, growth and development. An update to the 2007 review. Ann. Bot. Ann. Bot. 111, 1021–1058.
Yang, X., Hu, R., Yin, H., Jenkins, J., Shu, S., Tang, H., Liu, D., Weighill, D.A., Yim, W.C., Ha, J., Heyduk, K., Goodstein, D.M., Guo, H.B., Moseley, R.C., Fitzek, E., Jawdy, S., Zhang, Z., Xie, M., Hartwell, J., Grimwood, J., Abraham, P.E., Mewalal, R., Beltrán, J.D., Boxall, S.F., Dever, L.V., Palla, K.J., Albion, R., Garcia, T., Mayer, J.A., Lim, S.D., Wai, C.M., Peluso, P., Van Buren, R., De Paoli, H.C., Borland, A.M., Guo, H., Chen, J.G., Muchero, W., Yin, Y., Jacobson, D.A., Tschaplinski, T.J., Hettich, R.L., Ming, R., Winter, K., Leebens-Mack, J.H., Smith, J.A.C., Cushman, J.C., Schmutz, J., Tuskan, G.A., 2017. The *Kalanchoë* genome provides insights into convergent evolution and building blocs of crassulacean acid metabolism. Nat. Commun. 8, 1899.
Yu, K., D'Odorico, P., Carr, D.E., Personius, A., Collins, S.L., 2017. The effect of nitrogen availability and water conditions on competition between a facultative CAM plant and an invasive grass. Ecol. Evol. 7, 7739–7749.
Zaharia, A., Zaharia, D., Cantor, M., Buta, E., 2010. Research regarding the effects of water retention mechanisms, resistance to drought, heatstroke and high temperatures of the *Sempervivum* rosettes. Analele Universitatii Din Craiova 90, 568–573.
Zhao, Y., Chan, Z., Gao, J., Xing, L., Cao, M., Yu, C., Hu, Y., You, J., Shi, H., Zhu, Y., Gong, Y., Mu, Z., Wang, H., Deng, X., Wang, P., Bressan, R.A., Zhu, J.-K., 2016. ABA receptor PYL9 promotes drought resistance and leaf senescence. Proc. Natl. Acad. Sci. USA 113, 1949–1954.
Zuur, A., Ieno, E., Elphick, C., 2009. A protocol for data exploration to avoid common statistical problems. Methods Ecol. Evol. 1, 3–14.