

Research paper

Effects of drought and increased temperature on phytochemical traits of the edible halophyte *Crithmum maritimum*: Perspectives for future climatic scenarios

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ABSTRACT

Climate change, characterised by drought events and rising temperatures, exerts a significant threat to crop productivity and global food security. Halophytes, known for their resilience in harsh conditions, offer promising options for sustainable cultivation alternatives. Our study focused on *Crithmum maritimum*, commonly known as sea fennel, an edible halophyte with potential in the food and nutraceutical industries, to explore the impacts of drought and increased temperatures on its nutritional and antioxidant profiles. Different *C. maritimum* populations displayed high nutritional qualities, suitable for consumption despite appearing slight differences among localities. While both drought and increased temperatures affected plant growth and phytochemical profiles, their impact on nutritional value was minor. Surprisingly, drought induced an unexpected decline in phenolic content, challenging the assumption of increased antioxidants in response to water scarcity. Different rates of decrease in leaf production were observed among *C. maritimum* populations under drought, yet overall, they maintained similar levels, suggesting potential suitability for cultivation in environments with limited water availability. Diverse population-specific responses under climatic treatments revealed different alterations in amino acid and oxidative stress profiles, suggesting diverse adaptive strategies. These findings provide critical insights into *C. maritimum* adaptability to climate-driven changes, offering valuable information for future agricultural practices

1. Introduction

Climate change, characterized by increased drought frequency and higher temperatures, is profoundly affecting global agricultural productivity and threatening global food security (FAO, 2023). Both drought and heat stress have detrimental effects on plant physiological processes, leading to oxidative stress, cell damage, reduced photosynthesis and productivity and, in severe cases, cell death (Guo et al., 2016; Giordano et al., 2021). This agricultural challenge, coupled with the depletion of freshwater sources and the increasing global food demand, underscores the need for innovative solutions to grow crops under

adverse environmental conditions. Halophytes, due to their exceptional ability to thrive in adverse environments, offer a promising alternative as cash crops due to their high resilience and great nutritional value with antioxidant metabolites (Ventura et al., 2015; Duarte et al., 2022).

Halophytic species have evolved multiple physiological adaptations to thrive in harsh environments, particularly in salinized soils with limited water availability (Duarte et al., 2020). For instance, they exhibit multiple drought-resistance mechanisms that safeguard cellular functioning under osmotic stress and ensure sustained growth (Slama et al., 2007; Duarte et al., 2020). Additionally, the exposure of halophytes to adverse environmental conditions derives in the synthesis of

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antioxidant metabolites (mainly phenolics) as a protective mechanism, giving these plants an added nutraceutical value (Ksouri et al., 2007; Carreiras et al., 2020). Consequently, halophytes potentially optimize their nutritional and functional value under severe abiotic situations while maintaining robust plant performance (Agudelo et al., 2021).

While previous studies have extensively explored the effects of salinity on the physiological responses and nutritional quality of halophytic plants (e.g. Katschnig et al., 2013; Bendaly et al., 2016; Martins-Noguerol et al., 2021), less information is available about the effects of drought and increased temperature (Qiu and Lu, 2003; Panda et al., 2021; Duarte et al., 2015) and scarce data are available on the combined effects of both abiotic stressors on these plants. Understanding the combined influence of interconnected climatic factors on these plants is crucial, as their interactive effects could significantly affect both productivity and phytochemical profiles (Prasad et al., 2011; Craufurd et al., 2013). Furthermore, there is a lack of information on how these abiotic factors could influence the nutritional value and production of antioxidant compounds in halophytes, including amino acid and fatty acid profiles.

Crithmum maritimum L. (Apiaceae), commonly known as sea fennel, is a halophyte species with promising applications in human consumption as well as a source of nutraceuticals (Karkanis et al., 2022). Its wide distribution across diverse habitats, such as sandy and rocky beaches, cliffs, and its natural tolerance to adverse environmental conditions highlights its potential as a sustainable crop in arid and semi-arid regions. This potential extends to the production of commercial products, particularly for food purposes, due to its rich nutritional properties, including minerals (Nabet et al., 2017), essential amino acids and essential fatty acids, and its ability to produce antioxidant compounds (Sánchez-Faure et al., 2020; Martins-Noguerol et al., 2022). *Crithmum maritimum* has shown significant variability in its nutritional and antioxidant profiles, influenced by factors such as geographic origin (Burczyk et al., 2002; Meot-Duros and Magné, 2009), seasonal fluctuations in the wild (Mekinić et al., 2018), genotypic differences (Kadoglidou et al., 2022), salinity (Ventura et al., 2014), and variations between cultivated and their wild counterparts (Martins-Noguerol et al., 2022). Moreover, in a previous study by our group we observed that *C. maritimum* alters its phytochemical profile in response to salinity, nutrient deprivation, and their combined effects (Castillo et al., 2022). Given the predicted increase in the frequency and intensity of extreme heatwaves and drought events (IPCC, 2021), it is imperative to investigate the nutritional and antioxidant profiles of *C. maritimum* under future climatic scenarios of higher aridity. In this study, we aim to unravel the intricate modifications induced by drought and increased temperature on the nutritional and antioxidant profiles of different populations of *C. maritimum*. We selected four populations spanning diverse latitudes to explore how adaptation to specific environments influences responses to common stressors. The specific objectives of this study were to evaluate: (i) differences in populations regarding yield and the quality of metabolites of nutritional interest; (ii) how populations vary their phytochemical composition and yield in response to drought and increased temperature; and (iii) whether there are interactive effects between the population of origin and climatic treatments on phytochemical composition. This study contributes to a better understanding of plant responses to changes in climatic conditions, but also holds promising implications for the advancement of sustainable cultivation practices and the use of halophytes to address the challenges driven by climate change.

2. Materials and methods

2.1. Plant material and experimental design

Seeds were collected from four wild populations of *C. maritimum* selected along the coasts of Europe to reflect the diversity of ecosystems and environmental conditions where the species grows: Vilanova

(Galicia, northwest Spain; 42.5603178/ -8.8309103), Roche (Cádiz, south Spain; 36.314138/ -6.153952), Stomio (south Greece; 39.862070/ 22.752138) and Newborough (west England; 53.144290/ -4.406627; Supplementary Figure 1). Seeds were sown in trays with wet sand and maintained at the greenhouse facilities of the University of Seville under natural daylight (minimum light intensity $200 \mu\text{mol m}^{-2} \text{s}^{-1}$ and maximum $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$), temperature between 22 and 25 °C (night/day) and 40–60 % relative humidity. The vapor pressure deficit in the greenhouse was maintained within a range of 1–1.5 kPa. Uniform seedlings were transferred to plastic pots (13.5 cm diameter x 18 cm height) using commercial washed sand (0.5–1 mm particle size) as substrate. Pots were maintained in trays and irrigated with 20 % Hoagland's solution (Hoagland and Arnon, 1938) to achieve optimal growth until the plants reached approximately 58 cm in height. Experimental plants were transplanted into plastic plots (15.5 cm diameter x 15 cm height) with bottom drainage holes using a mixture of sand:peat (2:1) and irrigated periodically. To ensure maximum nutrient supply, a controlled release fertilizer (Nutricote, Projar, Spain) was added following the manufacturer's indications. After 30 days of growth, the drought and temperature treatments were started. The experiment was arranged in a completely randomised design with two water supply regimes, 100 % field water capacity (non-water limiting) and 20 % field water capacity (drought), and two temperature regimes (22–25 °C and 25–28 °C, night/day). 8–10 replicates were used for each treatment. Thus, the experiment was conducted using fully crossed design comprising 4 populations x 2 temperatures x 2 water availability regimes x 8–10 replicates per treatment combination, resulting in 144 experimental pots. In the 144 study pots, soil water content was measured weekly using the time-domain reflectometry method (TDR), recording two measurements per pot. Temperature was monitored and verified daily using greenhouse monitoring systems. Additionally, trays and plant position within trays were relocated weekly in a randomized manner to mitigate potential environmental variability. The experiment was maintained for 14 weeks.

2.2. Measurement of growth and photosynthesis-related parameters

Initial dry biomass of a subset of 15 plants and final dry biomass of all plants were measured by harvesting, oven-drying at 80 °C for 48 h and weighing the dry weight (DW) at the beginning and end of the experiment (i.e. after the 14 weeks of treatment). At the end of the experiment, the dry biomass of leaves, stems and roots were recorded. The relative growth rate (RGR, $\text{g}^{-1} \text{d}^{-1}$) was calculated as follow: $\text{RGR} = (\ln B_f - \ln B_i) \cdot D^{-1}$, where B_f is the final dry mass, B_i the initial dry mass and D is the duration of experiment (days). Net photosynthetic rates were measured per plant in fully expanded leaves at the end of the experiment. At this point, the sea fennel plants were mature and actively photosynthesizing. Gas exchange measurements were taken in the morning between 10:00 AM and 12:00 AM to ensure the plants were photosynthetically active and conditions were stable. These measurements were taken on each individual plant. Gas exchange was measured with an infrared gas analyzer in an open system (LI-6400, LI-COR Inc., Neb., USA). Net photosynthetic rate (A) was determined at an ambient CO_2 concentration of $400 \mu\text{mol mol}^{-1}$, a photon flux density of $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$, and at 25–28 °C (according to chamber temperature) and 45–55 % relative humidity. A was calculated according to von Caemmerer and Farquhar (1981).

2.3. Quantification of malondialdehyde (MDA) and proline

Malondialdehyde (MDA) and proline were measured as indicators of plant stress. MDA is an oxidative stress metabolite and was quantified as a measure of the level of lipid peroxidation, using the thiobarbituric (TBA) reactive substances method (Hodges et al., 1999). The experiment was conducted with three replicates per population and treatment. Fresh tissue samples (0.1 g) were ground in 2 mL of 80 % ethanol, the

homogenate was soaked at room temperature for 1 hour and finally centrifuged at 12000 g for 5 min at 4 °C. The supernatant was used as crude extract. Aliquots of 0.3 mL of supernatant were added to 0.7 mL of TBA [5 % TBA (w/v) in 20 % TCA (trichloroacetic acid; w/v)] and to 0.7 mL of 20 % TCA. The mixture was heated at 95 °C for 30 min, and then cooled. After centrifugation at 12,000 g for 10 min, the absorbance of the supernatant was measured at 532 nm. MDA concentration was calculated using an extinction coefficient of 155 mM⁻¹ cm⁻¹. The value was corrected for non-specific absorption subtracting the absorbance obtained with TCA from that of TBA. Proline is a compatible osmolyte and osmotic stress metabolite that was quantified according to Pérez-López et al. (2023). For proline the same ethanolic crude extract that for MDA determination was used. Aliquots of 50 µL of ethanolic extracts were added to 100 µL of reaction mix [ninhydrin 1 % (w/v) in acetic acid 60 % (v/v), ethanol 20 % (v/v)] by well, mixed and heated at 95 °C, 20 min. After cooling at room temperature, 100 µL were transferred to microplate wells and absorbance at 520 nm read with a microplate reader. Proline concentration was determined from a standard curve in the range from 1 to 50 nmol of proline in which its relationship with the absorbance is linear. A new standard curve was made for every batch reagent.

2.4. Lipid extraction and fatty acids

Total lipids were extracted from samples (approximately 100 mg of lyophilized leaves) according to Hara and Radin (1978), by grounding them with 4 mL of 2-propanol in a glass homogenizer. These measurements were conducted in three replicates per population and treatment. The mixtures were heated at 80 °C for 15 min to inactivate phospholipases and increase the yield extraction. After cooling, 6 mL hexane were added to the samples and shaken vigorously, and then 5 mL sodium sulphate 6.7 % (w/v) were also added and mixed again. The mixture was centrifuged and the upper hexane-rich phase containing lipids was transferred to clean tubes. The aqueous phase was extracted again with 7.5 mL of hexane:2-propanol (7:2, v/v), and the upper phase was extracted and combined with the previously obtained. Fatty acids methylation was performed by adding 3 mL methanol:toluene:sulphuric acid (88:10:2, v/v/v) to the lipid samples and the fatty acid methyl esters (FAMES) were extracted twice with 1 mL heptane and analysed by gas-liquid chromatography (GLC) using a Hewlett Packard 6890 gas chromatograph (Palo Alto, CA, USA) and a Supelco SP-2380 capillary column (60 m length, 0.25 mm i.d., 0.20 µm film thickness; Supelco, Bellefonte, PA, USA). Hydrogen was used as the carrier gas at 20 cm/s, the temperature of the flame ionization detector and injector was 220 °C, the oven temperature was 185 °C and the split ratio was 100:1. Heptadecanoic acid (17:0; Sigma-Aldrich, Missouri, USA) was used as internal standard for fatty acids quantification. The area of the peaks was determined as final step of the peak integration using ChemStation V.B04 software (Agilent, Santa Clara, USA). The % values reported were determined as % of each peak respect to total area detected. We calculated the total percentage of polyunsaturated fatty acids (PUFA) as the sum of the peaks corresponding to hexadecadienoic, hexadecatrienoic, linoleic and linolenic acids; the total percentage of unsaturated fatty acids (UFA) as the sum of palmitoleic, hexadecadienoic, hexadecatrienoic, petroselinic, oleic, linoleic and linolenic acids; and the total percentage of saturated fatty acids (SFA) as the sum of palmitic, stearic, arachidic and behenic acids. The percentage of essential fatty acids in the human diet was calculated as the sum of linoleic (18:2) and linolenic (18:3) fatty acids.

2.5. Determination of protein and amino acid composition

The protein content of leaves was determined in finely ground lyophilized biomass by elemental microanalysis of nitrogen content (x 6.25) with a LECO CN-828 analyzer (St. Joseph, MI, USA). The experiment was conducted with three replicates per population and treatment.

The amino acid contents were assessed according to Alaiz et al. (1992). Samples (4–6 mg of proteins) were hydrolyzed with 4 mL of HCl 6 N at 110 °C for 24 h in tubes under nitrogen atmosphere. Then, samples were dried using a rotary evaporator and then diluted in 10 mL of sodium borate 1 M pH 9.0. Derivatization process was performed using diethyl ethoxymethylenemalonate (Sigma Chemical Co., Missouri, USA) at 50 °C for 50 min. Separation of amino acids was developed by ultra-performance liquid chromatography (UPLC) using a reverse phase column (XSelect HSS T3 2.5 µm of 3.0 × 150 mm, Waters, Massachusetts, USA) in a binary gradient system with 25 mM sodium acetate 0.02 % (w/v) sodium azide pH 6.0 (Buffer A) and acetonitrile (Buffer B) as solvents. The elution and operate conditions were set according to Martins-Noguerol et al. (2023). D, L-α-aminobutyric (Sigma Chemical Co., Missouri, USA) was used as an internal standard for amino acid quantification using calibration curves obtained for each one. The tryptophan content was determined in samples of 20 mg of proteins that were hydrolyzed with 3 mL of NaOH 4 N at 110 °C for 4 h in tubes under inert nitrogen atmosphere according to Yust et al. (2004). Accordingly, samples were neutralized with HCl and completed with 1 M sodium borate buffer pH 9.0 up to 10 mL. Tryptophan was quantified by UPLC using a reverse phase column (XSelect HSS T3 2.5 µm of 3.0 × 150 mm, Waters, Massachusetts, USA) using as elution solvents the buffers A:B (91:9) in a elution flow of 0.8 mL/min and 25 °C of analytical temperature. Total essential amino acids (in the human diet) were calculated as the sum of His, Thr, Arg, Tyr, Val, Met, Cys, Ile, Trp, Leu, Phe and Lys (in % on total amino acids).

2.6. Mineral composition in plant leaves

Leaf samples were washed with 1 % HCl diluted in water and deionized water, oven-dried at 70 °C for 48 h, and then ground using a plant grinder. These measurements were conducted in three replicates per population and treatment. Samples of approximately 0.5 g DW were weighed directly into Teflon vessels. Accordingly, 4 mL NHO₃ suprapur (Tracepure™ 140 HNO₃; Merck, New Jersey, USA) were added to the samples and they were shaken gently. Samples were then subjected to microwave digestion (START D Microwave Digestion System, Milestone, Sorisole, Italy). After cooling, the digests were diluted with ultrapure water (< 18 MΩ/cm) up to 50 mL and they were passed through nylon filters (0.45 µm). The foliar concentrations of mineral elements were analysed by inductively coupled plasma optical emission spectroscopy, ICP-OES, with a Varian ICP 720-ES (Agilent Technologies, Inc., Santa Clara, CA, USA). The operating conditions for ICP-OES were set according to Martins-Noguerol et al. (2022). Yttrium (Y) 1000 mg/L (Merck, New Jersey, USA) was used as internal standard. The accuracy and precision of method were confirmed by standard reference material (*Brassica oleracea* sample from Plant-analytical Exchange (IPE) international program, Wageningen Evaluating Programmes for Analytical Laboratories, WEPAL). Calibration curves were performed in 8 % HNO₃ with the multi-elemental standards Certipur solution (Merck, New Jersey, USA) and Spectrascan certified reference solution (LGC Standards GmbH, Wesel, Germany) and the phosphorus mono-elemental standard for its calibration curve.

2.7. Phenolic composition

Phenolic compounds were extracted from 20 mg of dried leaf material with 0.25 mL of 70 % methanol in an ultrasonic bath for 15 min, followed centrifugation, the extract was filtered through a 0.20-µm micropore polytetrafluoroethylene (PTFE) membrane and placed in vials for chromatographic analysis (Moreira et al., 2021). Measurements were taken for each plant in all populations and treatments. Chemical identification of the polyphenol composition was performed using an ultra-performance liquid chromatography coupled with electrospray ionization quadrupole (Thermo Dionex Ultimate 3000 LC) time-of-flight mass spectrometry (UPLC-Q-TOF-MS/MS) (Compact™) (Bruker

Table 1

Phytochemical traits of different *C. maritimum* populations (Roche, Stomio, Newborough and Vilanova) according to linear models: protein content (% dry weight), essential fatty acid proportion (% on total fatty acids), total phenolic content (TPC, mg/g DW), phenolic acid and flavonoid proportions (% on TPC), and total concentration of Ca, K, Mg, P (expressed in percentage, g/100 g DW), Fe, Mn, Zn, Cu and Sr (expressed in mg/kg DW) in leaves. Data represent average and standard error of 12 independent replicates. Different letters indicate significant differences ($p < 0.05$) according to linear models.

	Units	Roche	Stomio	Newborough	Vilanova
Protein	%	15.97 ± 1.48	15.29 ± 1.07	16.34 ± 1.07	15.60 ± 1.12
Essential fatty acids	%	54.56 ± 1.18a	64.31 ± 0.77b	58.98 ± 1.04ab	59.44 ± 0.75a
TPC	mg/g DW	81.55 ± 12.89	119.40 ± 18.88	142.63 ± 25.21	124.91 ± 22.08
Phenolic acids	%	78.35 ± 12.39	91.23 ± 14.42	93.98 ± 16.61	91.32 ± 16.14
Flavonoids	%	21.65 ± 3.42	8.77 ± 1.39	6.02 ± 1.06	8.68 ± 1.53
Ca	g/100 g DW	3.00 ± 0.11a	3.09 ± 0.13a	3.00 ± 0.09a	3.58 ± 0.07b
K	g/100 g DW	2.13 ± 0.13a	2.63 ± 0.15b	2.23 ± 0.14a	2.11 ± 0.12a
Mg	g/100 g DW	0.30 ± 0.01a	0.22 ± 0.02b	0.24 ± 0.01b	0.30 ± 0.02a
P	g/100 g DW	0.37 ± 0.02a	0.36 ± 0.01a	0.51 ± 0.03b	0.45 ± 0.03b
Al	mg/kg DW	9.78 ± 1.11	14.46 ± 1.48	13.79 ± 0.98	12.18 ± 1.25
Cu	mg/kg DW	3.13 ± 0.13	3.48 ± 0.56	2.96 ± 0.07	3.44 ± 0.36
Sr	mg/kg DW	34.73 ± 1.68ab	32.21 ± 2.12b	30.51 ± 1.67b	38.10 ± 1.57a
Zn	mg/kg DW	16.24 ± 1.56	17.37 ± 1.81	17.42 ± 1.42	16.56 ± 1.54

Daltonics GmbH, Bremen, Germany). Chromatographic separation was developed in a Kinetex™ 2.6 μm C18 82–102 Å, LC Column 100 × 4.6 mm column with a binary gradient solvent mode consisting of 0.05 % formic acid in water (solvent A) and acetonitrile (solvent B). The gradient used was the following: from 10 % to 30 % B (0–5 min), from 30 % to 50 % B (5–10 min), from 50 % to 100 % B (10–12 min), hold 100 % B until 14 min, from 100 % to 10 % B (14–15 min), hold 10 % B until 17 min. The injection volume was 3 μL , the flow rate was established at 0.4 mL/min and column temperature was controlled at 35 °C. MS analysis was operated in a spectra acquisition range from 50 to 1200 m/z . Negative (-) ESI modes were used under the following specific conditions: gas flow 8 L/min, nebulizer pressure 38 psi, dry gas 7 L/min, and dry temperature 220 °C. Capillary and end plate offset were set to 4500 and 500 V, respectively. MS/MS analysis was performed based on the previously determined accurate mass and RT and fragmented by using different collision energy ramps to cover a range from 15 to 50 eV. Individual compounds were identified on the basis of the data obtained from the standard substances or published literature, including RT, λ_{max} , ([M-H]⁻), and major fragment ions. For the quantitative analysis of phenolic compounds, 10 μL of each sample was then analysed using the same column and conditions described previously, in an UHPLC (Nexera LC-30CE; Shimadzu, Tokio, Japan) with a Nexera SIL-30AC injector and one SPD-M20A UV/VIS photodiode array detector (Shimadzu, Tokio, Japan); see [Moreira et al. \(2021\)](#) for more details of the chromatographic analyses. Chromatograms were recorded at 330 nm. The flavonoids were quantified as rutin equivalents and hydroxycinnamic acids as chlorogenic acid equivalents. We achieved the quantification of these phenolic compounds by external calibration using calibration curves at least with six data points, from 0.01 to 1 mM. Caffeoyl quinic acids and p-coumaroyl quinic acids derivatives were quantified as chlorogenic acid (hydroxycinnamic acids) (Sigma–Aldrich Chemie GmbH, Steinheim, Germany), flavonoids were quantified as rutin (Sigma–Aldrich Chemie GmbH, Steinheim, Germany). The limits of detection and quantification for the compounds were in the range of 0.3 and 1 ng for chlorogenic acid and 0.6 and 1 ng for rutin. The recoveries of compounds were calculated in three different concentrations in the range of 93.7–104.1 %. Total phenolic content was calculated as the sum concentration of each individual compound.

2.8. Statistical analyses

Statistical analyses were performed using R software version 4.1.1 ([R Core Team, 2021](#)). We conducted linear models to evaluate the independent effects of geographic origin, the climatic factors and their interactive effects on the growth, photosynthesis, plant stress (i.e. MDA, proline) and phytochemical profile (i.e. lipids, fatty acids, protein, amino acids, minerals and phenolic compounds) of *C. maritimum* plants.

Each individual pot was considered a biological replicate. When significant interactions between temperature and water treatments were found, post-hoc Tukey pairwise comparisons were performed. All variables were tested for normality using the Shapiro-Wilk test and for homoscedasticity with the Levene's test, and they were square-root-, inverse-, log-, Box-Cox or logit-transformed when necessary to meet assumptions of the analysis.

3. Results

3.1. Differences among *C. maritimum* populations

Linear models indicated that all plants exhibited a consistent protein content ranging from 15.3 % to 16.3 % across populations ([Table 1](#); [Supplementary Table 1](#)). Stomio plants displayed the highest essential fatty acid proportion, although not significantly different when compared to Newborough plants ([Table 1](#)). Moreover, uniform antioxidant content was observed, ranging from 81.6 to 142.6 mg phenolics/g DW, with similar proportions of phenolic acids and flavonoids ([Table 1](#)). Regarding leaf mineral composition, Vilanova plants exhibited significantly higher levels of Ca and slightly higher proportions of Sr compared to some populations ([Table 1](#)).

Additionally, while the lipid profiles were uniform across the populations, Roche plants notably exhibited the highest content of petroselinic acid ([Supplementary Table 2](#)). In terms of antioxidant composition, all plants showed similar phenolic compounds and profiles ([Supplementary Table 3](#)). However, Roche plants produced higher proportion of two feruloylquinic acids, one isomer of coumaroylquinic acid, and an isomer of caffeoylcoumaroylquinic acid, along with the lowest proportion of a caffeic acid isomer.

3.2. Effects of drought and increased temperature

Both climatic factors, drought and increased temperature, significantly altered plant biomass production, with drought having a more pronounced effect. Increased temperature significantly reduced root growth, but did not affect aerial (leaves and stems), leaf or stem biomass ([Fig. 1 A](#); [Supplementary Table 4](#)). Conversely, drought significantly decreased aerial, leaf, root, and stem biomass ([Fig. 1 A](#)). Linear models indicated interactive effects of both climatic factors, with their combined influence leading to an additional 12 % decrease in leaf biomass ([Fig. 1 B](#)). Otherwise, RWC and net photosynthesis rate were not significantly affected by climatic treatments. Specifically, net photosynthesis remained between 9.8 and 16.7 $\mu\text{mol m}^{-2} \text{s}^{-1}$ across climatic treatments. Specifically, only drought induced a significant increase in proline and MDA content ([Supplementary Figure 2](#)), indicative of higher osmotic and oxidative stress in plants, respectively.

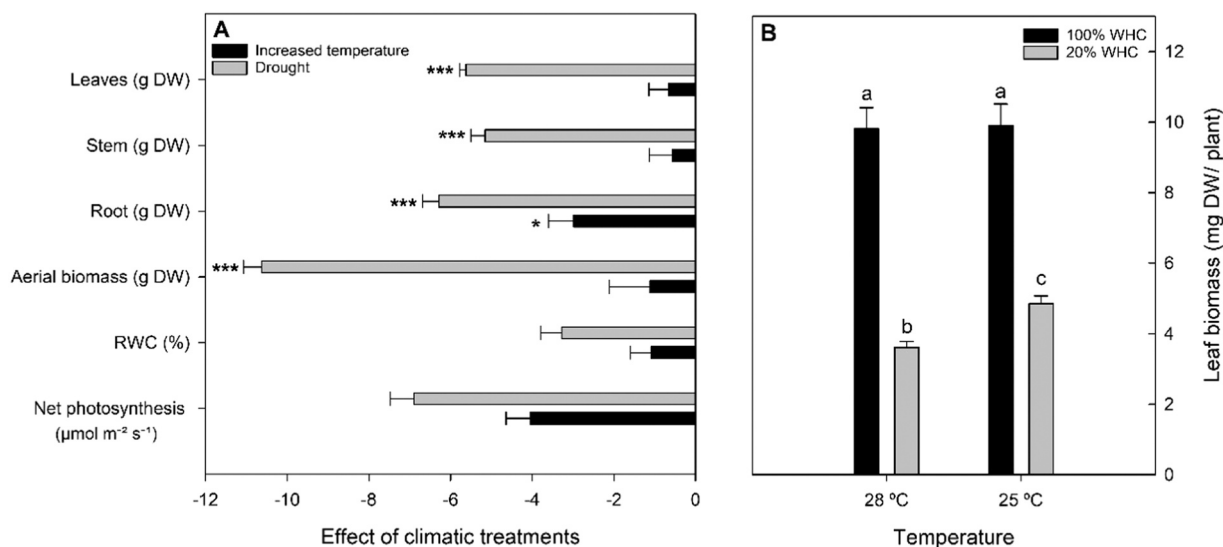


Fig. 1. (A) Effects of drought and increased temperature (with respect to control conditions) on *C. maritimum* plant performance: leaf, stem, root and total aerial biomass (g DW/ plant), relative water content (%) and net photosynthesis rate ($\mu\text{mol m}^{-2} \text{s}^{-1}$). Data represent average and standard error of 72 independent replicates. Significant effects according to linear models are indicated with (*) ($p < 0.05$) and (***) ($p < 0.001$). (B) Interactive effects of climatic treatments (100 % and 20 % whc and 25 °C and 28 °C) on leaf biomass (g DW/ plant). Data represent average and standard error of 36 independent replicates. Different letters indicate significant differences ($p < 0.05$) according to the linear models.

Furthermore, both climatic factors significantly affected the phytochemical composition of *C. maritimum* (Fig. 2). Drought caused a significant 7.1 % increase in protein content, but resulted in a significant 6.1 % decrease in the proportion of essential amino acids. It also decreased total phenolic content (TPC) by 57 %, along with a 0.1 % reduction in lipid content. Increased temperature significantly reduced lipid content and essential fatty acids by 0.1 % and 4.5 %, respectively (Fig. 2).

Both treatments also affected mineral accumulation, both macro- (Fig. 3A) and micronutrients (Fig. 3B); drought significantly decreased S, Na, and B accumulation while increasing Zn content in *C. maritimum* leaves, whereas increased temperature significantly increased Zn content.

Additionally, both climatic factors significantly affected the amino acid composition of leaves, with drought exerting a stronger impact by increasing Glu proportion and decreasing Ala, Ile, Trp, Leu, Phe, and Lys. In contrast, increased temperature augmented Pro proportion and reduced Trp (Fig. 4).

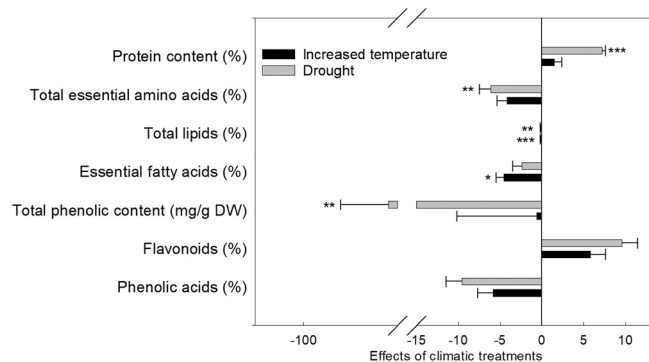


Fig. 2. Effects of drought and increased temperature on quality and antioxidant traits of *C. maritimum* leaves: protein content (% on DW), total essential amino acids (% on total amino acids), lipid content (% on DW), essential fatty acids (% on total lipids), total phenolic content (% on DW), flavonoids (% on TPC), phenolic acids (% on TPC). Data represent average and standard error of 24 independent replicates. Significant effects according to linear models are indicated with (*) ($p < 0.05$), (**) ($p < 0.01$), (***) ($p < 0.001$).

Likewise, drought exerted a stronger impact on phenolic composition than increased temperature (Supplementary Figure 3). It significantly increased the proportion of feruloylquinic acid 1 and rutin while decreasing the proportion of two coumaroylquinic acid isomers and two caffeic acid isomers. Meanwhile, increased temperature only slightly increased feruloylquinic acid 1.

In addition, significant interactions between drought and increased temperature were observed for several phytochemical traits (Fig. 5; Supplementary Tables 2, 5–7). B content was significantly reduced by drought but only at 25 °C, whereas the negative effects of increased temperature only appeared under optimal humidity conditions (Fig. 5A), and Fe, Mn, and Mg content increased due to their combined effects (Fig. 5B–D). The proportion of a caffeic acid isomer was only significantly reduced by drought without increasing temperature (Fig. 5E), while feruloylquinic acid 2 proportion was significantly increased due to the combined effects of both climatic factors (Fig. 5F). Changes in the fatty acid profile were evident only when both climatic stressors operated simultaneously, affecting the proportion of the two major fatty acids of *C. maritimum* leaves (Supplementary Table 6); linoleic acid tended to increase under simultaneous drought and increased temperature conditions, while linolenic acid was significantly reduced with increased temperature and this decrease was more marked under both climatic stressors operating simultaneously (Fig. 5G–H). Consequently, the linoleic/linolenic ratio was significantly increased when both climatic stressors were present (Fig. 5I).

3.3. Interactive effects of drought, increased temperature and population on plant performance and phytochemical profile

The linear models revealed significant differences in the responses of the different *C. maritimum* populations to both drought and increased temperature in terms of biomass production and phytochemical traits, especially amino acid composition. While drought induced a reduction in aerial biomass (both in leaf and stem) in all *C. maritimum* populations, interactive effects of drought and the population were observed, particularly indicating that Roche and Vilanova displayed a greater decrease in aerial biomass production, both leaf and stem, per plant (approximately 62 % less leaf biomass) compared to the other populations (with a 49 % reduction; Fig. 6).

Moreover, although significant effects of drought and increased

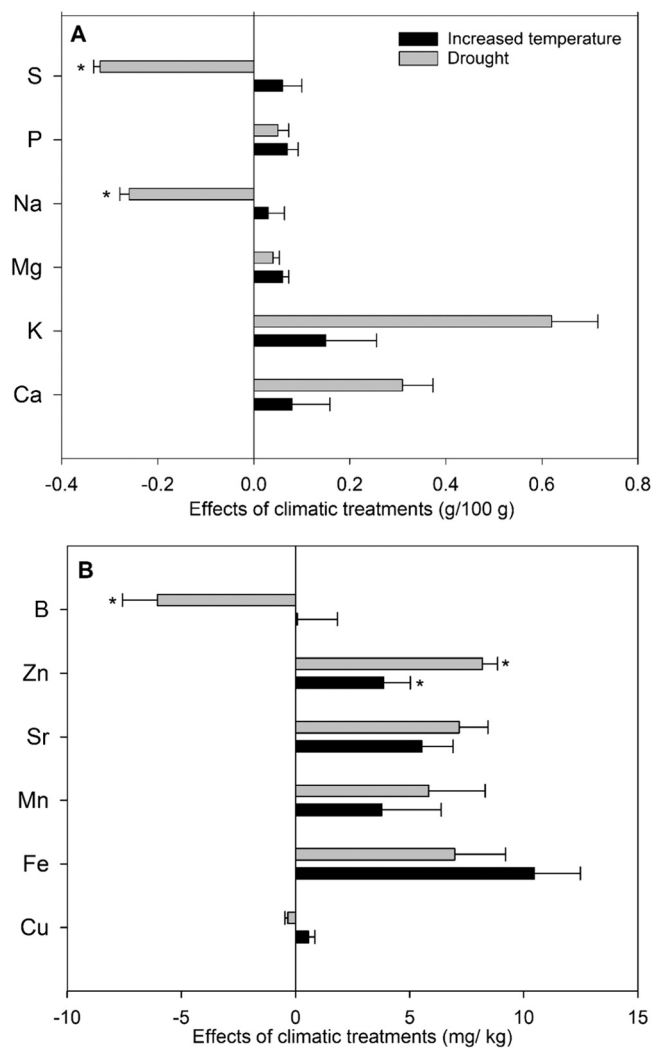


Fig. 3. Effects of drought and increased temperature on mineral accumulation of *C. maritimum*. (A) Macro-nutrients and sodium (g/ 100 g DW). (B) Micro-nutrients (mg/kg DW). Data represent average and standard error of 24 independent replicates. Significant effects according to linear models are indicated with (*) ($p < 0.05$).

temperature on amino acids were evident, significant interactions emerged depending on the population (Fig. 7; Supplementary Table 5). For example, under increased temperature, only Roche and Vilanova plants decreased the proportion of Trp, with Roche also displaying a reduction in His, while Newborough plants increased Cys (Fig. 7A). Conversely, under drought conditions, Roche, Newborough, and Vilanova plants showed an increase in Glu and a decrease in Leu and Ile, while only Roche plants showed a decrease in Ser, His, Thr and Gly proportions under this climatic treatment (Fig. 7B).

Despite these changes, all populations under both drought and increased temperature exhibited an amino acid profile generally aligned with FAO guidelines (FAO, 2002), except for the sulfur amino acids (Cys and Met) and Trp (Supplementary Tables 8–9). Finally, other significant interactions were observed in Na, B and essential amino acid accumulation, although the effects were minimal (Supplementary Figure 4). Notably, although not statistically significant, there was a trend towards lower MDA levels at 25 °C and 100 % WHC in all plants except those from Roche, which displayed the lowest levels at 28 °C and 100 % WHC (Supplementary Figure 5).

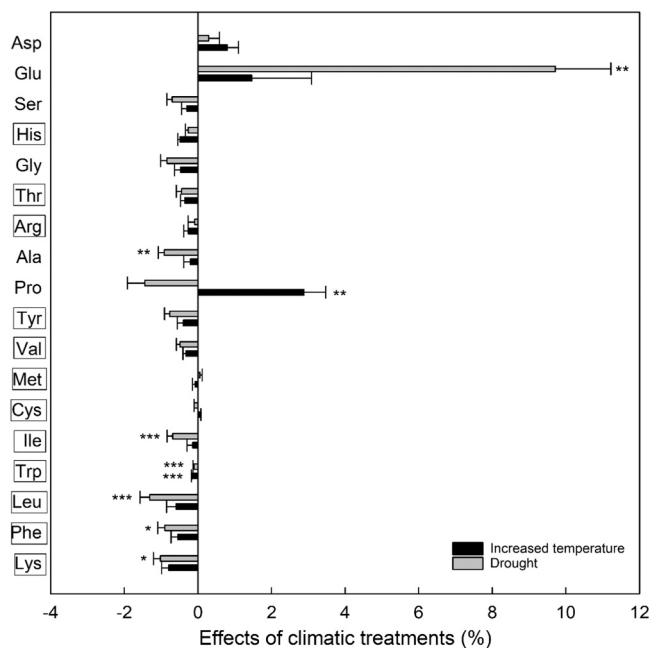


Fig. 4. Effects of drought and increased temperature on amino acid composition (% on total amino acids) of *C. maritimum* leaves. Essential amino acids are boxed. Data represent average and standard error of 24 independent replicates. Significant effects according to linear models are indicated with (*) ($p < 0.05$), (**) ($p < 0.01$), (***) ($p < 0.001$).

4. Discussion

4.1. Variability among *C. maritimum* populations

Our findings revealed a consistent nutritional profile of *C. maritimum* suitable for human consumption, although with significant differences among the study populations. Notably, protein remained consistent and even exceeded the levels reported for other edible halophytes, such as *Sarcocornia perennis* and *Salicornia ramosissima* (Barreira et al., 2017). The lipid content, rich in essential fatty acids such as linoleic and α -linolenic acids, emphasizes the different *C. maritimum* populations from commonly consumed leafy vegetables such as lettuce or spinach (Simopoulos, 2004). Moreover, the high PUFA/SFA ratio aligns with nutritional guidelines (WHO/FAO, 2003), highlighting the potential of *C. maritimum* as a viable dietary option. Notably, Vilanova plants exhibited the highest accumulation of Ca and Sr, suggesting that this population may serve as a superior source of these minerals. Furthermore, Roche plants displayed elevated levels of petroselinic acid, valued in functional food, pharmaceutical, and nutraceutical industries (Delbeke et al., 2016), enhancing the overall value of this population exploitation. The observed differences in nutrient and phytochemical profiles among populations, despite cultivation under uniform greenhouse conditions, suggest a strong genotypic influence on their metabolic composition.

Surprisingly, the TPC and phenolic profile remained remarkably uniform among populations despite previously reported variability in content and composition based on population and geographic origin (Burczyk et al., 2002; Meot-Duros and Magné, 2009; Kadoglidou et al., 2022). The uniform profile of these secondary metabolites across populations in our study could be attributed to standardized growth conditions and harvest times. However, slight but significant differences indicated that Roche plants had higher proportion of coumaroylquinic and feruloylquinic acids, which are recognized for their health benefits (Vanderhaegen et al., 2006). All four populations exhibited higher TPC levels compared to those reported for French (23–33 mg gallic acid equivalent/g DW; Meot-Duros and Magné, 2009) or Greek

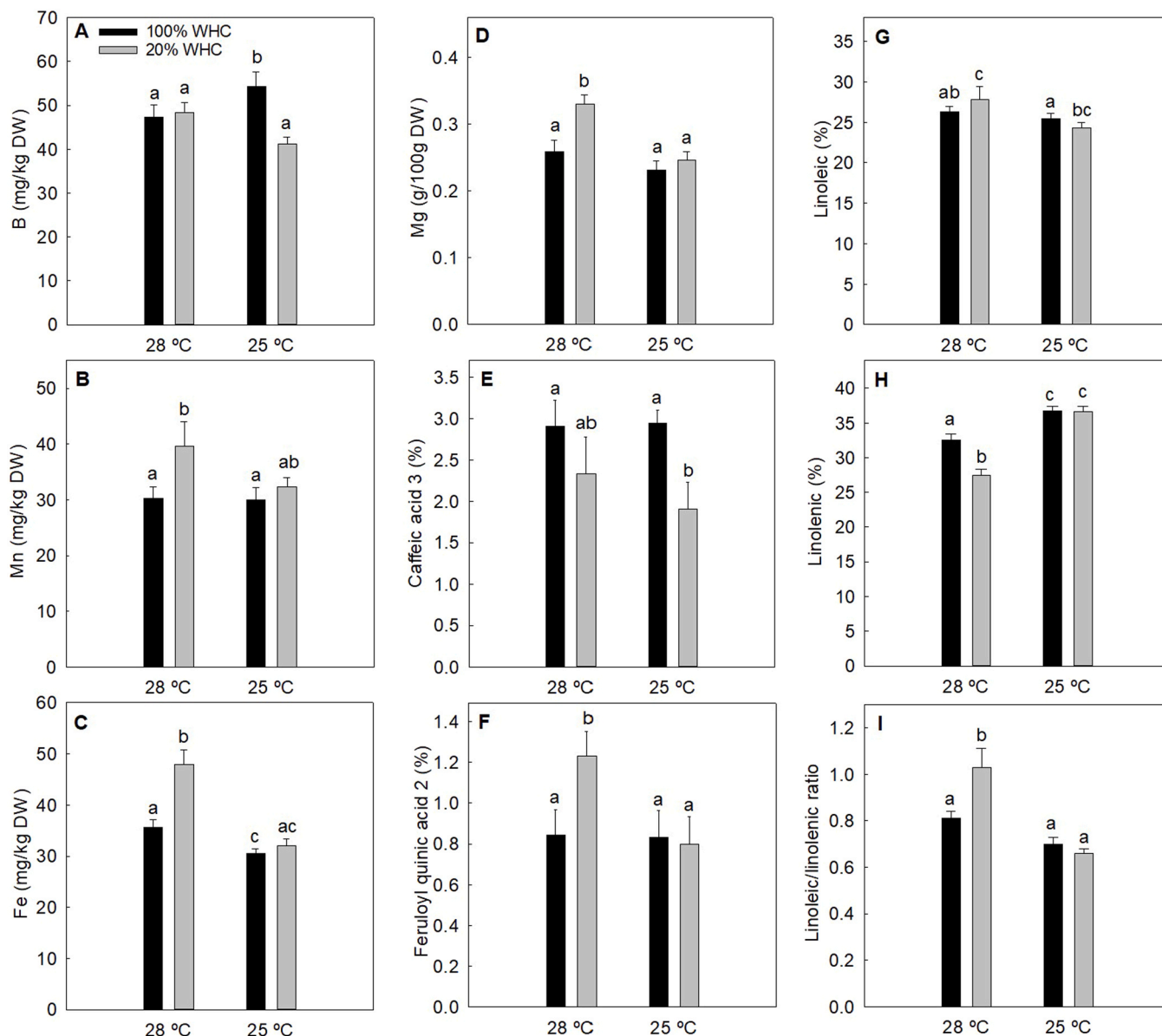


Fig. 5. Interactive effects of drought and increased temperature on the phytochemical traits of *C. maritimum* leaves according to linear models. Data represent average and standard error of 12 (minerals and fatty acids) and 36 (phenolics) independent replicates. Significant differences ($p < 0.05$) from linear models are indicated with different letters.

(2.55–10.84 mg GAE/g DW; Kadoglidou et al., 2022) *C. maritimum* populations and other halophytes (Ksouri et al., 2007), suggesting their potential as valuable sources of phenolics for various commercial applications. Our observations highlight the importance of considering these wild varieties for cultivation and the need for further investigations to optimize secondary metabolite accumulation in cultivated *C. maritimum*.

4.2. Effects of climatic treatments

The significant reduction in plant biomass under drought and increased temperature could be interpreted as an adaptive strategy to conserve energy and water in response to the abiotic stressors (Muller et al., 2011). Increased temperature tended to decrease the overall growth of *C. maritimum*, but this reduction was significant only in roots, a pattern previously observed in other plant species (Huang and Xu, 2000; Calleja-Cabrera et al., 2020). Drought, however, had a more pronounced impact, reducing both aerial and belowground biomass,

which is consistent with previous studies on other halophytes such as *Haloxylon salicornicum*, *Salicornia brachiata* and *Salvadora persica* (Parida and Jha, 2013; Rangani et al., 2018; Panda et al., 2021). Drought affects cell elongation and expansion, which restricts plant growth, biomass accumulation and productivity (Farooq et al., 2009).

Reduced growth is often correlates with water retention via stomatal closure and the limitation of reactive oxygen species (ROS) accumulation through antioxidant mechanisms (Begum et al., 2019; Kaya et al., 2020). Consistently, leaf RWC in *C. maritimum* was unaffected by drought, suggesting the maintenance of tissue water status, likely due to stomatal closure and reduced transpiration, as observed in other halophytes (Parida and Jha, 2013; Panda et al., 2021). Despite a trend toward decreased photosynthetic rates under drought and increased temperature, no significant alterations in net photosynthesis were observed. This contrasts with findings in other halophyte species (Rangani et al., 2018; Lu et al., 2021), highlighting the relevance of this species as a future tolerant crop under sub-optimal environmental conditions.

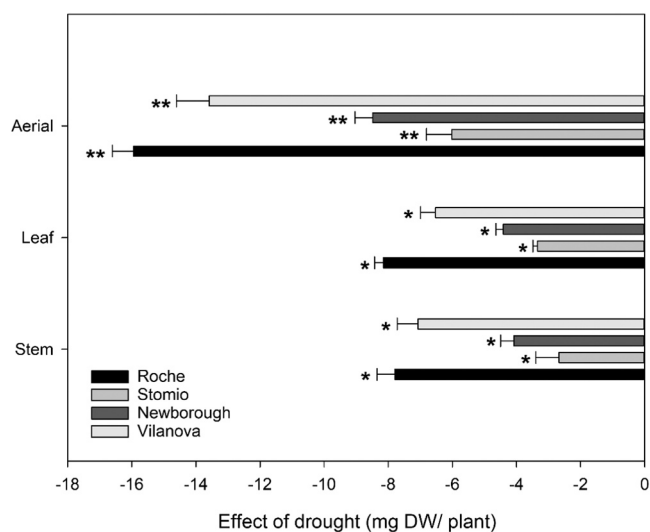


Fig. 6. Effects of increased drought on aerial biomass production of the different *C. maritimum* populations. Data represent average and standard error of 16–20 independent replicates. Significant effects of drought according to linear models are indicated with (*) ($p < 0.05$) and (**) ($p < 0.01$).

Reduced *C. maritimum* growth under drought was coupled to an increased MDA and proline accumulation, indicating drought and oxidative stress (Demidchik, 2015). MDA, a byproduct of lipid peroxidation due to oxidative damage (Sgherri et al., 2018), increased under drought, similar to other halophytes such as *Halogeton glomeratus* (Lu et al., 2021). Proline, an osmolyte that is accumulated in response to drought and salt stress, also increased, as observed in *H. glomeratus* (Lu et al., 2021) or *Salicornia brachiata* (Parida and Jha, 2013). However, the lower increase in MDA levels suggests that *C. maritimum* maintains cell homeostasis through efficient osmoprotective and antioxidant mechanisms. While proline contributes to drought tolerance, its low concentration indicates other mechanisms may be involved in the osmotic adjustment, warranting further investigation. Additionally, while the combined effects of both climatic factors significantly reduced leaf production by 12 %, no interactions between these factors were observed concerning RWC, photosynthesis, MDA, or proline. These results suggest that *C. maritimum* might tolerate the combination of both abiotic factors.

The influence of both drought and increased temperature on the phytochemical composition of *C. maritimum* was evident, with drought showing a stronger effect. Drought conditions increased protein content, a known drought tolerance mechanism linked to stress-induced protein synthesis, essential for maintaining cell osmotic potential and physiological status (Li et al., 2010; Mi et al., 2022). However, the decrease in essential amino acids suggests a trade-off between protein synthesis and resource reallocation under stress, which can consequently affect the protein quality. Alterations in the amino acid profile, such as the 10 % increase in Glu, a precursor in proline biosynthesis, indicate metabolic adaptations for stress resistance (Hildebrandt, 2018; Kiani-Pouya et al., 2017). Nonetheless, the decrease in amino acids such as Ala, Ile, Trp, Leu, Phe, and Lys under drought, and the minor shifts in Pro and Trp under increased temperature require further investigation to understand their roles in osmoprotection and dehydration tolerance (Panda et al., 2021; Haghghi et al., 2022).

Similarly, the reduction in lipid content and essential fatty acids (i.e. linoleic and linolenic acids) under elevated temperatures may represent an adaptive strategy for stress tolerance (Liu et al., 2019). Although decreases in total lipids are common in several plant species (Upchurch, 2008), the modest 0.1 % reduction in *C. maritimum* suggests a limited impact despite severe water deprivation, potentially due to inhibited desaturase activity, a common adaptation to water deficit

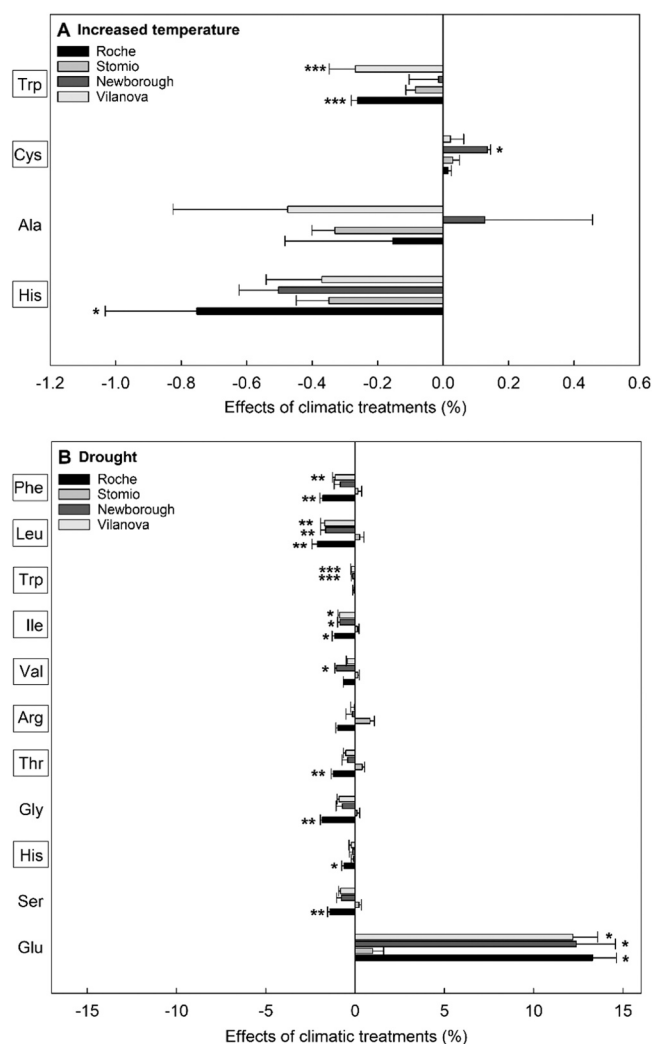


Fig. 7. Effects of increased temperature (A) and drought (B) on amino acid composition (% on total amino acid) of the different *C. maritimum* populations. Essential amino acids are boxed. Data represent average and standard error of 6 independent replicates. Significant effects according to linear models are indicated with (*) ($p < 0.05$), (**) ($p < 0.01$) and (***) ($p < 0.001$).

(Torres-Franklin et al., 2009). Under combined stressors, linoleic acid levels increased while linolenic acid was notably reduced, raising the linoleic/linolenic ratio, consistent with adaptations in halophytes during osmotic stress (Liu et al., 2019; Duarte et al., 2020). Despite reductions due to drought, these essential omega-6 and omega-3 fatty acids, remained high at 26 % and 32 %, respectively, of total fatty acids, emphasizing their health benefits (Loconsole et al., 2019).

Moreover, shifts in mineral content under drought conditions might result from altered availability and competitive uptake within the plant organs (Rangani et al., 2020). The decrease in S, B and Zn content could be attributed to their limited availability and altered uptake dynamics, down-regulating their assimilation pathways in leaves (Marschner, 1995; Ahanger et al., 2016). However, major elements such as Na, Ca, and Mg, which are crucial for osmoprotection and membrane stability (Parida and Jha, 2013), remained unaffected by climatic factors. This suggests efficient regulation of mineral uptake and distribution as a drought tolerance strategy, as in other halophytes (Parida and Jha, 2013; Rangani et al., 2018; Panda et al., 2021). The stability of most micronutrient levels further indicates the plant's adaptability in regulating mineral uptake under stress.

The stronger impact of drought on the phenolic composition, mainly by reducing TPC and the phenolic acid proportion, contrast with typical

response in other halophytes, where polyphenols often increase under drought (Abd El-Maboud, 2019; Parida and Jha, 2013). However, most studies on drought in halophytes utilized less severe water restrictions and shorter durations (e.g., 30 % field capacity for 14 days in *Salvadora persica* or 30 % PEG-treated plants for 7 days in *Salicornia brachiata*; Parida and Jha, 2013; Rangani et al., 2018). Long-term and severe drought stress may inhibit the synthesis of phenolic compounds in *C. maritimum* leaves, reallocating resources to sustain vital functions, consistent with findings in non-halophyte species (Naikoo et al., 2019; Król et al., 2014; Aninbon et al., 2016). Despite the reduction in TPC, intriguing modifications in the phenolic profile under combined stressors suggest potential adaptations to simultaneous environmental challenges.

The overall non-significant effect of increased temperature on many variables could be due to the specific temperature range applied in this study. Temperature effects can be highly variable depending on the magnitude of the increase and the species (Wahid et al., 2007). Therefore, the moderate temperature increase used in our study could be within the tolerance range of *C. maritimum*, with non-significant changes in many phytochemical traits, in contrast to the pronounced impact of drought treatment. Further studies should investigate higher temperatures to elucidate the thresholds leading to significant physiological and biochemical variations.

4.3. Interactive effects of drought, increased temperature and population on plant traits

Our study reveals significant interactions between drought and *C. maritimum* populations, highlighting varying responses among them. Plants from Roche and Vilanova showed the most substantial decrease in leaf biomass under drought, suggesting differential susceptibility to water stress, which may be attributed to inherent genetic differences influencing water use efficiency and stress tolerance mechanisms. Likewise, distinct patterns in nutritional composition were observed, especially in the accumulation of specific amino acids, suggesting population-specific responses to abiotic factors.

Despite variations in amino acid proportions among populations, they generally aligned with FAO guidelines (FAO, 2002), except for sulfur amino acids (Cys and Met) and Trp, similar to other edible halophytes such as *Halimione portulacoides* (Martins-Noguerol et al., 2021). Different plant genotypes or populations have shown great variations in their amino acid composition (Aloisi et al., 2016; Jekabsons et al., 2022). The observed interactions between populations and climatic factors that influence the accumulation of Na, B, or essential amino acids underscore substantial diversity in their responses, yet without a distinct pattern or significant impact on nutritional value. Likewise, distinct lipid peroxidation patterns were observed in *C. maritimum* plants from Roche, suggesting different levels of oxidative stress. Previous studies on halophytes reported variations in stress tolerance and metabolic responses among different populations (Purmale et al., 2022; Ievinsh, 2023), which supports our findings. However, previous research has mainly focused on salinity effects, making this study the first to compare the impact of drought and increased temperature on different populations of a wild edible halophyte, particularly regarding nutritional traits. These population-specific responses could stem from inherent genetic differences (Simopoulos et al., 2020), emphasizing the role of genetic variability in determining plant adaptability to stress.

A remarkable similarity in the nutritional and antioxidant profiles of geographically distant *C. maritimum* populations suggests low genetic diversity within the species (Kadoglidou et al., 2022). However, previous studies reported significant differences in phytochemical composition among different genotypes of *C. maritimum*, even those in closer geographic proximity (Kadoglidou et al., 2022). Despite significant geographic distances, minor changes in phytochemicals were observed, minimally affecting the overall nutritional value of these populations under drought and increased temperature. These results prompt a

re-evaluation of the assumptions concerning the expected diversity in phytochemical profiles across distant populations, particularly when they are grown under identical conditions. Further exploration into underlying mechanisms could provide insights into species' resilience and adaptability to environmental challenges, valuable for domestication and integration into various agroecological areas.

5. Conclusions

Our study reveals a consistent nutritional profile among diverse populations of *C. maritimum*, despite significant impact from climatic treatments, particularly drought. Adaptive changes in nutrient traits induced by treatments had minimal effect on overall nutritional value. The unexpected reduction in phenolic content under drought emphasizes the negative impact of limited water availability on the antioxidant properties. Despite geographic separation, the interactions between treatments and populations suggest minor variations in nutritional and functional value, suggesting a consistent phytochemical profile. This uniformity offers potential for species domestication and integration into diverse agroecological contexts, providing essential insights into species' resilience mechanisms under predicted climate change scenarios.

CRedit authorship contribution statement

Marta Francisco: Methodology, Investigation. **Enrique Martínez-Force:** Writing – review & editing, Resources, Methodology. **Justo Pedroche:** Resources, Methodology. **Jacinto Gandullo:** Writing – review & editing, Methodology, Investigation. **Rosario Álvarez:** Writing – review & editing, Methodology, Investigation. **Luis Matías:** Writing – review & editing, Validation, Software, Methodology, Formal analysis, Conceptualization. **Diego Rico-Jiménez:** Software, Investigation, Formal analysis. **Jesús Cambrollé:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization. **Raquel Martins-Noguerol:** Writing – review & editing, Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Data curation. **Antonio J. Moreno-Pérez:** Writing – review & editing, Validation, Methodology, Investigation. **Xoaquín Moreira:** Writing – review & editing, Resources, Methodology, Investigation. **Ignacio M. Pérez-Ramos:** Writing – review & editing, Validation, Conceptualization.

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.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.envexpbot.2024.105924](https://doi.org/10.1016/j.envexpbot.2024.105924).

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