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Diverse projected climate change scenarios affect the physiology of broccoli plants to different extents

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Abstract

Climate change caused by global warming involves crucial plant growth factors such as atmospheric CO_2 concentration, ambient temperature or water availability. These stressors usually co-occur, causing intricate alterations in plant physiology and development. This work focuses on how elevated atmospheric CO_2 levels, together with the concomitant high temperature, would affect the physiology of a relevant crop, such as broccoli. Particular attention has been paid to those defence mechanisms that contribute to plant fitness under abiotic stress. Results show that both photosynthesis and leaf transpiration were reduced in plants grown under climate change environments compared to those grown under current climate conditions. Furthermore, an induction of carbohydrate catabolism pointed to a redistribution from primary to secondary metabolism. This result could be related to a reinforcement of cell walls, as well as to an increase in the pool of antioxidants in the leaves. Broccoli plants, a C_3 crop, grown under an intermediate condition showed activation of those adaptive mechanisms, which would contribute to coping with abiotic stress, as confirmed by reduced levels of lipid peroxidation relative to current climate conditions. On the contrary, the most severe climate change scenario exceeded the adaptive capacity of broccoli plants, as shown by the inhibition of growth and reduced vigour of plants. In conclusion, only a moderate increase in atmospheric CO_2 concentration and temperature would not have a negative impact on broccoli crop yields.

KEYWORDS
Brassica, chlorophyll fluorescence imaging, high atmospheric CO_2, high temperature, hyperspectral reflectance imaging, multicolour fluorescence imaging, plant physiology, representative concentration pathway

1 | INTRODUCTION

Climate change, driven primarily by anthropogenic activities, is altering global environmental conditions at an unprecedented rate. Rising global temperatures and increased atmospheric CO_2 concentrations have far-reaching consequences for life on Earth, particularly in the Mediterranean region, which is considered one of the areas most affected by climate change (Gualdi et al., 2013). Indeed, irreversible weather fluctuations caused by climate change pose concerning scenarios for many sectors, mainly agriculture, as they threaten the production and supply of sufficient food worldwide (Abbass et al., 2022). As global temperatures rise and weather patterns become more

erratic, impacts on plant metabolism can be severe throughout the life cycle (Challinor et al., 2014), ranging from altered germination and slow vegetative growth (related to reduced photosynthesis and/or premature senescence) to altered or anomalous reproduction and seed development (Lippmann et al., 2019). With an increasing demand on the world market due to its high nutritional value, broccoli (*Brassica oleracea* var. *italica*), a C_3 plant, is one of the most important vegetable crops whose cultivation is nowadays facing numerous challenges due to climate change, as its growth requires moderate temperatures (Siomos et al., 2022). The main broccoli-producing area in Spain is Región de Murcia, in the Mediterranean basin, with a production of 25,1267.5 tons, 45% of the total Spanish production in 2021 (according to the Spanish Ministerio de Agricultura, Pesca y Alimentación; https://www.mapa.gob.es/es/estadistica/temas/estadistica-digital/powerbi-cultivos.aspx, accessed on 3rd November 2023).

Studying the impact of climate change on crop plants is vital for predicting and mitigating the impacts on agricultural production (Hatfield et al., 2011). Research provides insights into the mechanisms underlying plant responses, including physiological, biochemical, and molecular changes, and enables the identification of key targets for plant adaptation strategies. To date, it is known that climate change will alter agricultural activities in areas growing brassicas such as white cabbage (*B. oleracea* var. *capitata*), as the maximum length of the growing season will be extended (Čimo et al., 2020). Alterations in brassicas metabolism caused by high temperatures and high atmospheric CO_2 levels could be behind this phenomenon. Indeed, heat stress and/or rising CO_2 concentrations are known to impair redox metabolism to different extents depending on the weather conditions and the plant analysed, often damaging membranes and thus reducing photosynthesis and also affecting secondary metabolism and transpiration (Bita & Gerats, 2013, Dusenge et al., 2019). This was particularly true for oilseed rape plants (*B. napus* var. *napobrassica*) cultivated under two climate change scenarios described in the 5th Assessment Report (AR5) of the Intergovernmental Panel on Climate Change (IPCC, 2015): the intermediate and the extreme representative concentration pathways (RCP 4.5 and RCP 8.5, respectively). Oilseed rape plants grown at RCP 4.5 and RCP 8.5 displayed premature leaf ageing due to lipid peroxidation resulting in loss of chlorophyll (Chl) and accumulation of carotenoids (Car) and xanthophylls (Xanth). This led to downregulation of photosynthesis, accumulation of phenolic compounds and a decrease in leaf transpiration that was even more drastic in the case of oilseed rape plants grown at RCP 8.5 (Pineda & Barón, 2022). Other studies on *B. napus* cv. 45H72 cultivated on

conditions comparable to RCP 4.5 showed similar results (Qaderi et al., 2006).

Nowadays, imaging techniques are a very valuable tool to analyse, record and report plant fitness in crop fields, as they allow quantitative spatial and temporal analysis of plants physiology (Berger et al., 2022). Among these techniques, the most commonly used are: (1) thermography, a quick and reliable technique for indirectly quantifying leaf transpiration (Jones, 2004); (2) green fluorescence (F520), excited by UV light, emitted by phenolic compounds mostly bound covalently to cell walls (Buschmann & Lichtenthaler, 1998); (3) chlorophyll red fluorescence imaging (Chl-FI), emitted by Chl *a* and indicative of photosynthetic activity (Pérez-Bueno et al., 2019); and iv) light hyperspectral reflectance that allows the calculation vegetation indices (VIs), many of which have been related to physiological traits such as fitness, vigour, water content or pigment composition (Roberts et al., 2018).

In this work, the metabolic stress responses and fitness of broccoli plants have been analysed under two climate change scenarios relative to the current climate conditions (CCC). The forecasted conditions tested were RCP 4.5 and RCP 8.5 (Table 1). The capacity of broccoli plants to adapt to stressing ambient conditions would be key to plant fitness under future climate conditions. A moderate rise in ambient temperature and CO_2 concentration could be positive for photosynthetic activity and, subsequently, for plant growth and fitness. However, research is needed to learn to what extent these stressors could be detrimental to crop yields and to find strategies to mitigate the negative effects of climate change. The impact of high atmospheric CO_2 levels in combination with high temperature on plant health state was evaluated in terms of activity of the primary and secondary metabolism, as well as the plant oxidative stress. To add value to classical physiological measures, we have used a unique combination of imaging phenotyping techniques that support the results obtained with traditional measures.

2 | MATERIALS AND METHODS

2.1 | Broccoli cultivation

Broccoli plants (*Brassica oleracea* var. *italic* cv. *calabrese natalino*; Semillas Fitó, Barcelona, Spain) were grown as previously described by Pineda et al. (2022). Three environmental conditions were selected, as described in Table 1: CCC, RCP 4.5 and RCP 8.5 being CCC

TABLE 1 Broccoli growth conditions. CCC: current climate conditions; RCP 4.5 and RCP 8.5: the intermediate and the extreme climate change projection for Región de Murcia by years 2081–2100. ppm: parts per million. PPFD, photosynthetic photon flux density.

Climate conditions	Temperature (°C)		CO_2 (ppm)	Relative humidity (%)	PPFD (μmol photon $\cdot m^{-2} \cdot s^{-1}$)	Photoperiod (h)	
	Day	Night				Day	Night
CCC	31	17	408	65	200	16	8
RCP 4.5	34	20	650				
RCP 8.5	37	23	1000				

considered as the control treatment. For CCC, day and night temperatures correspond to the current average values during the growing season (summer) in Región de Murcia, the main Spanish broccoli producer. In the case of RCP 4.5 and RCP 8.5 treatments, ambient temperature and CO_2 concentrations were chosen according to the data regionalized by the AEMet (Spanish Meteorology Agency) for Región de Murcia in the years 2081–2100. For every experiment, seed germination and plant growth were carried out under the corresponding environmental conditions. Periodical watering of plants was carried out along experiments to ensure water availability at all times, regardless of the ambient conditions.

2.2 | Quantification of leaf oxidative stress, phenolics and pigments

Sampling was done using $4.15\,cm^2$ leaf disks ($1.54\,cm^2$ in the case of broccoli plants grown at RCP 8.5) practised on leaf number 4 of broccoli plants after four (CCC and RCP 4.5) or five (RCP 8.5) weeks from sowing. Twelve different leaves were sampled and weighted for every measurement of plants grown under CCC and RCP 4.5 treatments, whereas ten plants were sampled in the case of plants grown under RCP 8.5. Sample weights were used to calculate fresh weight (FW) per leaf area. Once collected, samples were immediately frozen in liquid nitrogen and kept at $-80°C$ until the moment they were processed, according to Pineda & Barón (2022). Spectrophotometric determinations were carried out using the Shimadzu UV1800 spectrophotometer (Shimadzu Corporation). All the determinations (oxidative stress, soluble phenolics and pigments) are referred to FW.

The oxidative stress of leaves was evaluated by determining the total antioxidant activity (TAA) and lipid peroxidation, according to Miller et al. (1995) and Rodríguez-Serrano et al. (2016), respectively.

The total content of soluble phenolics, ortho-diphenols, flavonoids and phenylpropanoid glycosides (PPGs) were determined according to Chun & Kim (2004), Maestro-Durán et al. (1991), Zhishen et al. (1999) and Gálvez et al. (2005), respectively.

The leaf content on Chl (*a* and *b*), Car and Xanth were determined spectrophotometrically according to Lichtenthaler & Buschmann (2001).

2.3 | Metabolic profiling by gas chromatography followed by mass spectrometry (GS-MS)

GS-MS measurements were carried out by the Scientific Instrumentation Service of the Estación Experimental del Zaidín – CSIC (Granada, Spain), according to Liu et al. (2018). As in the case of colourimetric reactions, sampling was done using six leaf disks ($4.15cm^2$ for plants grown at CCC and RCP 4.5; $1.54cm^2$ in the case of plants grown at RCP 8.5) practised in leaf number four of twelve different broccoli plants (ten plants in the case of those grown under RCP 8.5). Samples were weighted, ground to a powder in liquid nitrogen and kept at $-80°C$ until the moment they were processed. Quantified metabolites

were: (1) the acids: quinic, caffeic, ferulic, citric, malic and succinic; (2) the amino acid phenylalanine; (3) the soluble sugars: glucose, fructose, sucrose and myo-inositol (Glc, Fru, Suc and MI, respectively); and (4) the tricarboxylic acids from Krebs cycle: citric, malic and succinic. Quantification was made using the Varian (now Bruker Corporation, Billerica, MA, USA) 450GC 240MS system for GC–MS (Ibort et al., 2017). Measurements are referred to FW.

2.4 | Plant phenotyping by imaging techniques

All measurements with image sensors were performed on attached leaves of broccoli plants, particularly the fourth true leaf. Images representing a collection of parameters were calculated following the equations in Table 2. The mean values of the different parameters were extracted for each whole leaf. Graphs show average values ± standard errors of thirty-six leaves per treatment (n = 36), except for chlorophyll fluorescence imaging (Chl-FI), with eighteen leaves per treatment (n = 18).

The photosynthetic performance in terms of photosystem II (PSII) activity was studied by variable Chl-FI, using an Open FluorCam 700 MF (Photon System Instruments) operated by FluorCam v. 5 software (Photon Systems Instruments). F_V/F_M, Φ_{PSII} and NPQ (maximum quantum yield of PSII, the effective quantum yield of PSII and non-photochemical quenching, respectively; Table 2) were recorded following protocol one previously described by Pineda et al. (2008).

FLIR A305sc camera (FLIR Systems) operated by FLIR ResearchIR v. 3.4 software was used to capture thermal images of whole leaves as described before (Pineda et al., 2022). The parameter $T_L - T_A$ (leaf temperature corrected by ambient temperature; Table 2) was calculated for a better comparison between climatic treatments.

The green autofluorescence at 520 nm (F520) was recorded using a customized Open FluorCam FC 800-O (Photon Systems Instruments), according to Pérez-Bueno et al. (2016). Values of F520 (Table 2) were obtained using FluorCam v. 7.1.0.3 software (Photon Systems Instruments).

Hyperspectral reflectance in the 700–1000 nm spectral range was recorded using a Pika L hyperspectral imaging camera (Resonon, Bozeman) operated by Spectronon v. 2.134 (Resonon) software, and according to Pineda et al. (2022). Reflectance spectra were obtained to calculate the VIs displayed in Table 2.

2.5 | Data mining and statistics

Statistical analysis data plotting was carried out using SPSS v. 28.0.1.0 (142) (IBM Corp.). First of all, data were represented in box plots to remove outliers (software). Then, to ensure comparison between climatic treatments, all collected data were normalized from zero to one according to the equation: *normalized value = (x-minimum value) / (maximum value-minimum value)*. As a result of the normalization, the data shown in the graphs are expressed in arbitrary units (A.U.). Table S1 shows the average values ± standard errors before

TABLE 2 Parameters obtained from broccoli leaf images and used in this work. Chl-FI: chlorophyll fluorescence imaging; F_0: minimum fluorescence in the dark-adapted state; F520: green fluorescence; F_M: maximum fluorescence in the dark-adapted state; F_M': maximum fluorescence in the light-adapted state; F_t: steady-state fluorescence; F_V: variable fluorescence; MCFI: multicolour fluorescence imaging; R_{xxx}: reflectance at the given wavelength; T_A: ambient temperature; T_L: leaf temperature.

Imaging technique	Parameter	Related to	Equation	References
Chl-FI	Maximum quantum yield of PSII	Photosynthesis	$F_V/F_M = (F_M - F_0)/F_M$	Pérez-Bueno et al. (2019)
	Effective quantum yield of PSII	Photosynthesis	$\Phi_{PSII} = (F_M' - F_t)/F_M'$	
	Non-photochemical quenching	Photosynthesis	$NPQ = (F_M - F_M')/F_M'$	
Thermography	Leaf temperature corrected by ambient temperature	Transpiration	$T_L - T_A$	Jones, 1999
MCFI	Green fluorescence	Secondary metabolism	F520	Buschmann & Lichtenthaler, 1998
Hyperspectral reflectance imaging	Anthocyanins reflectance index 1	Pigments	$ARI = (1/R_{550}) - (1/R_{700})$	Roberts et al. (2018)
	Vogelmann red edge index 1	Fitness and vigour	$VREI = R_{740}/R_{720}$	
	Water band index	Water content	$WBI = R_{900}/R_{970}$	
	Climatic stress index for brassicas	Climatic stress	$CSIB = R_{525}/R_{800}$	Pineda & Barón (2022)

normalization. Finally, statistical analysis was carried out to compare between treatments. Sample distributions were analysed using the Shapiro–Wilk normality test and Barlet-Levene equal variance test. Then, one-way ANOVA and Kruskal-Wallis tests were applied, when appropriate, to compare between treatments. Whenever a one-way ANOVA was applied, statistical groups were determined according to Tukey or T3 Dunnett test as needed. Differences were considered significant at $p < 0.05$ and were indicated by different letters in the graphs.

3 | RESULTS

3.1 | Broccoli development under climate change conditions

The combination of heat and elevated atmospheric CO_2 concentrations altered the morphology of broccoli plants. The plant size was inversely proportional to the severity of the environmental conditions (Table 1). Indeed, the growth of plants cultivated under RCP 8.5 was much slower than those grown in the other climatic treatments, producing fewer, smaller and curlier leaves during the same time span (Figure 1A). In order to compare plants at the same developmental stage regardless of the growth conditions, plants cultivated under RCP 8.5 were analysed when they were one week older than those grown under CCC or RCP 4.5 conditions. Thus, the fourth leaf, at the fully expanded stage, was analysed for every plant after four (CCC and RCP 4.5) or five (RCP 8.5) weeks after sowing. Additionally, the leaves of plants grown under extreme condition appeared thicker than the control leaves. Indeed, the FW per leaf area ratio, indicative of leaf thickness under no water deficiency (Afzal et al., 2017), showed the highest values for leaves of RCP 8.5 plants. On the contrary, the leaves of plants grown at RCP 4.5 had a lower FW per leaf area than the leaves of CCC plants (Figure 1B).
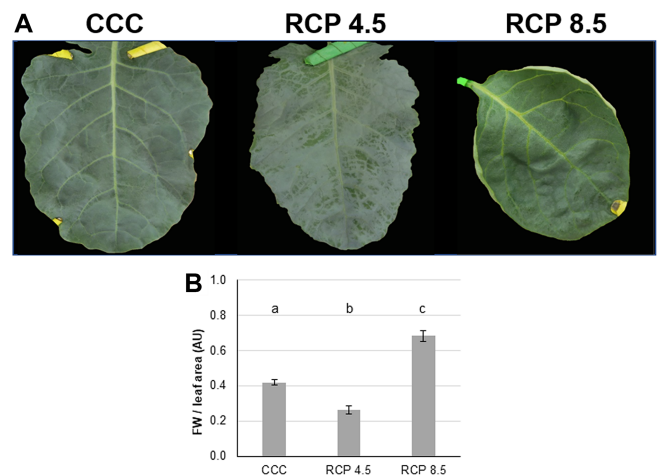

FIGURE 1 Symptoms induced by growing conditions on broccoli plants. **A)** RGB images showing the fourth leaf of plants grown under current climate conditions (CCC), intermediate and extreme climate change projections (representative concentration pathways (RCPs) 4.5 and 8.5, respectively). Plants were four (CCC and RCP 4.5) or five (RCP 8.5) weeks old. **B)** Normalized values of fresh weight (FW) measured per leaf area. Graphs show average values ± standard errors (n = 40 [RCP 8.5] or 48 [the rest] disks from different leaves). Lowercase letters indicate statistical differences at $p < 0.05$.

3.2 | Plant primary metabolism and pigment content

A collection of measurements was performed to assess the impact of climate change on the main metabolic process of broccoli plants. Regarding photosynthesis (Figure 2A; Table 2) the parameter F_V/F_M, which measures the maximum capacity of PSII to develop photosynthesis (Pérez-Bueno et al., 2019), showed no significant differences between the three climatic treatments, indicative of no permanent damage on the photosynthetic machinery. However, Φ_{PSII} (Table 2),
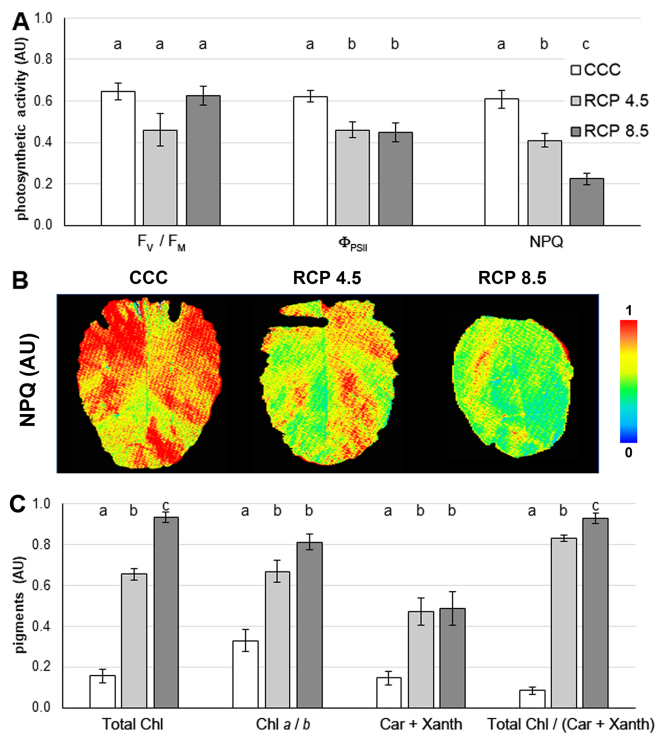
FIGURE 2 Primary metabolism of broccoli plants (I). **A)** Photosynthetic efficiency of whole leaves in terms of: maximum quantum yield of photosystem II or PSII (F_V/F_M), and effective quantum yield of PSII in the steady state (Φ_{PSII}) and non-photochemical quenching (NPQ) in the steady state. Graph show average normalized values ± standard errors (n = 18 whole leaves). **B)** Representative NPQ images of whole broccoli leaves. A false colour scale is applied. **C)** Pigment composition of broccoli leaves referred to fresh leaf weight. Graphs show average normalized values ± standard errors (n = 10 [RCP 8.5] or 12 [CCC and RCP 4.5] disks from different leaves). Lowercase letters indicate statistical differences at $p < 0.05$. Car: carotenoids; Chl: chlorophylls; Xanth: xanthophylls.

which quantifies the actual photosynthesis rate, showed a lower rate of electron transport in the thylakoids of leaves grown under climate change conditions relative to controls (Figure 2A). Similarly, the activity of energy dissipation mechanisms measured by the NPQ (Figure 2A, B; Table 2), is reduced in plants growing under climate change conditions, especially for those grown at RCP 8.5. Alterations in the photosynthetic activity were accompanied by alterations in the pigment composition of broccoli leaves (Figure 2C). The total Chl content was increased in leaves of plants grown at RCP 4.5 and RCP 8.5, along with an increment in the Chl a / b ratio compared to control plants. Furthermore, Car and Xanth were also increased in the leaves of plants grown in both climate change treatments. However, the total Chl / (Car + Xanth) ratio increased for both treatments compared to the control plants, which were more severely under RCP 8.5. These results point to changes in the composition of photosystems I and II and their antennae to adjust the activity of the electron transport of the thylakoid to the ambient conditions (Pinnola & Bassi, 2018).

The inhibition of the electron transport chain in the thylakoids correlated with alterations in carbohydrate metabolism (Figure 3A).

The Suc accumulation levels were significantly reduced in plants grown at RCP 4.5, and more severely at RCP 8.5, whereas the Glc content was decreased to a similar extent under RCP 4.5 and RCP 8.5, relative to CCC. However, Fru levels were not significantly affected by the ambient conditions. Thus, the ratio Fru / Glc increased by 4-fold relative to control plants, whereas the ratio Suc / (Glc + Fru) followed the opposite trend, showing statistically significant difference between plants grown under the two RCPs (Figure 3B). Furthermore, the alteration on the carbohydrate metabolism affects also the accumulation levels of another non-structural carbohydrate, MI, which serves as substrate to diverse biosynthesis pathways (Alok et al., 2022). MI decreased to the same extent for plants grown at RCP 4.5 and RCP 8.5 relative to control plants (Figure 3A).

Leaf transpiration, with an indirect impact on photosynthetic activity, can be estimated in terms of leaf temperature (Chaerle et al., 2005). Thus, T_L-T_A inversely correlates with leaf transpiration, which was higher under climate change projections relative to CCC (Figure 3C, D). However, no significant differences could be found between broccoli leaves of plants grown at RCP 4.5 and RCP 8.5 in terms of T_L-T_A.

Regarding respiration, the activity of the Krebs cycle was evaluated by quantifying the accumulation of some of its intermediates (tricarboxylic acids; Figure 3E). Particularly, citric and malic acid were decreased to the same extent in leaves of plants grown both at RCP 4.5 and RCP 8.5, compared to those grown at CCC; whereas the succinic acid was not affected by the growing conditions.

3.3 | Oxidative stress and plant secondary metabolism

TAA and lipid peroxidation of leaves grown under RCP 4.5 and RCP 8.5 decreased compared to those grown at CCC. However, lipid peroxidation was significantly higher for plants grown in the extreme climatic condition than for those grown at RCP 4.5 (Figure 4A). The antioxidant activity is partly due to the non-enzymatic antioxidants, which are mainly phenolic compounds derived from the secondary metabolism, particularly their soluble fraction (Podsędek, 2007). As with TAA, the total soluble phenolics content decreased under climate change conditions (Figure 4B); however, it decreased more drastically in the case of plants cultivated under RCP 4.5. Among the soluble phenolic compounds tested, ortho-diphenols and flavonoids accumulated to lower extents in plants grown under RCP 4.5 or RCP 8.5, relative to those grown at CCC. However, PPGs accumulation levels significantly decreased only for leaves of plants cultivated at RCP 4.5.

The autofluorescence at 520 nm (parameter F520, Table 2) is mainly emitted by phenolic compounds covalently bound to cell walls, including lignins, which are normally much more abundant than soluble phenolics (Buschmann & Lichtenthaler, 1998). F520 values were significantly lower for plants grown under RCP 4.5 but increased by 6-fold in those cultivated at RCP 8.5, relative to control plants (Figure 5A, B). These results suggest opposite effects on the extent of
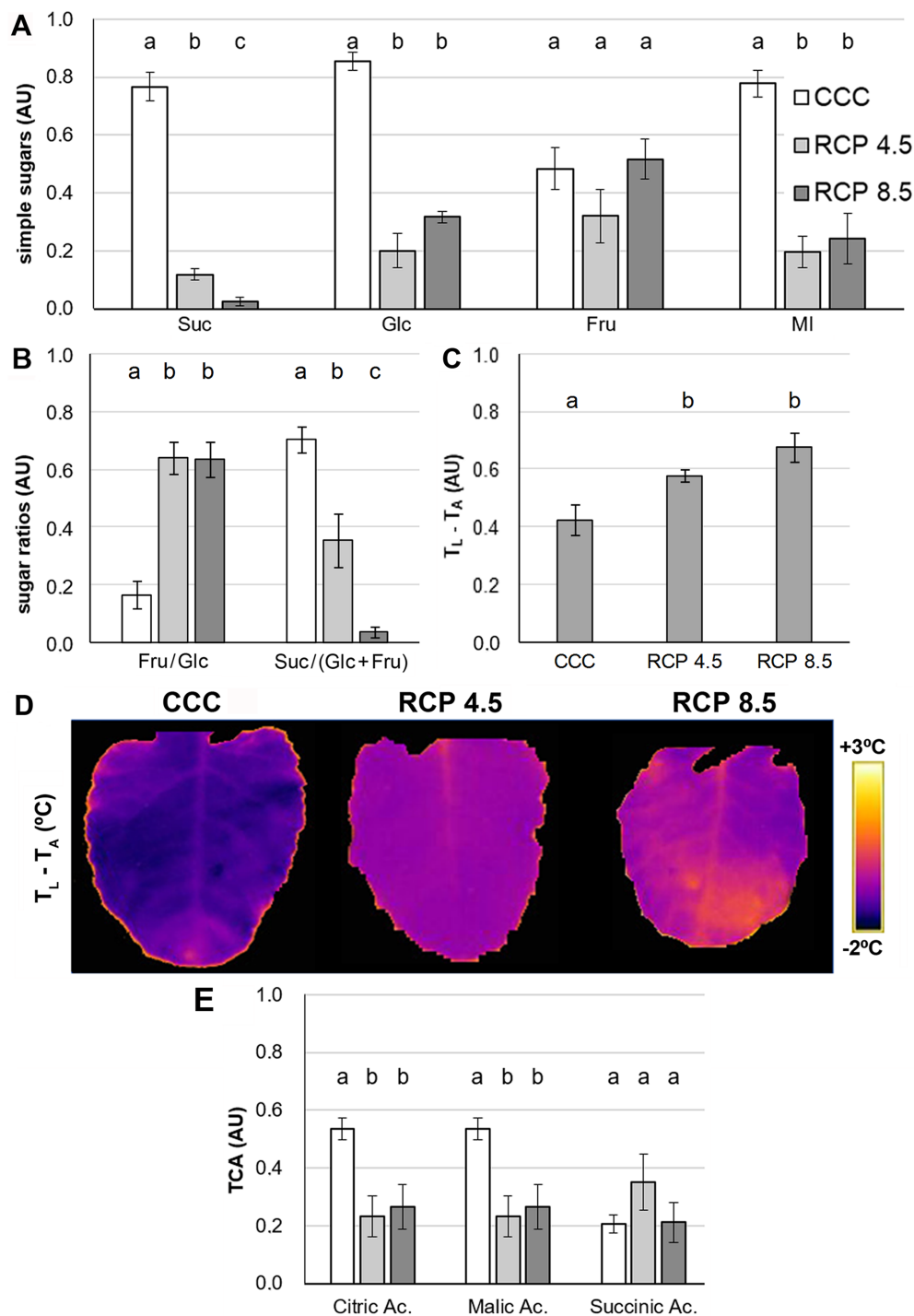
FIGURE 3 Primary metabolism of broccoli plants (II). **A)** Simple sugars composition in broccoli leaves, referred to fresh leaf weight. **B)** Sugar ratios. **C)** Leaf-air temperature differences (T_L-T_A). **D)** Representative thermal images of whole broccoli leaves. A false colour scale is applied. **E)** Content of different tricarboxylic acids of the Krebs cycle, referred to fresh weight. Graphs show average normalized values ± standard errors. For graphs in panels **A)**, **B)** and **E)** n = 10 (RCP 8.5) or 12 (the rest) disks from different leaves. In the case of panel **C)** n = 36 whole leaves. Lowercase letters indicate statistical differences at $p < 0.05$. Ac.: acid; Fru: fructose; Glc: glucose; MI: myo-inositol; Suc: sucrose.

cell wall lignification depending on the severity of environmental conditions.

Furthermore, phenylalanine is a key substrate for the main biosynthetic pathways of secondary metabolism (Dias et al., 2016). This amino acid was found to accumulate at higher levels in RCP 8.5 plants. A collection of secondary metabolites, key to plant stress response, were also analysed. Thus, a drastic decrease in the accumulation levels of quinic, caffeic and ferulic acids was found for plants cultivated under climate change conditions relative to control plants, with no significant differences between plants grown at RCP 4.5 and RCP 8.5 (Figure 5C).

3.4 | Vegetation indices

Several commonly used VIs are known to correlate with physiological traits such as plant fitness, vigour or pigment content (Roberts et al., 2018). That is the case of the VIs shown in Table 2 (and Figure 6): the anthocyanins reflectance index 1 (ARI 1), the Vogelmann red edge index 1 (VREI 1), the water band index (WBI) and the climate stress index for brassicas (CSIB). ARI 1, which correlates with the contents of anthocyanins, increased only in plants grown under the more severe RCP, proving to be a more stressful condition.
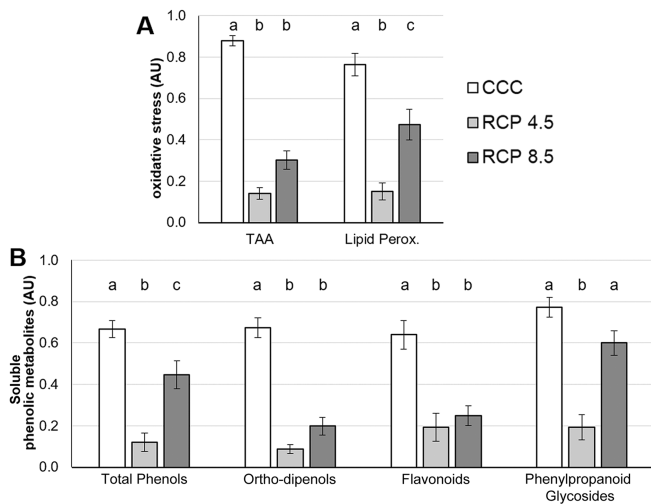
FIGURE 4 Oxidative stress and secondary metabolism of broccoli plants (I). **A)** Total antioxidant activity (TAA) and lipid peroxidation (lipid perox.) of broccoli leaves referred to fresh leaf weight. **B)** Content on soluble phenolic compounds in those leaves: total phenolics, orto-diphenols, flavonoids and phenylpropanoid glycosides referred to fresh leaf weight. Graphs show average normalized values ± standard errors (n = 10 [RCP 8.5] or 12 [rest of treatments] disks from different leaves). Lowercase letters indicate statistical differences at $p < 0.05$.

Similarly, the plant fitness estimated by VREI 1 significantly decreased under RCP conditions, particularly under RCP 8.5. Following the same trend, the parameter WBI, related to the water content of leaves, decreased compared to the control plants; however, the decrease was significant only in the case of plants grown at RCP 8.5. Additionally, the parameter CSIB (Pineda & Barón, 2022), indicative of abiotic stress in oilseed rape, increased when plants were cultivated under elevated temperature and CO_2, particularly under RCP 8.5 (Figure 6).

4 | DISCUSSION

The main aim of this work is to widen our knowledge about the impact of climate change on crops by combining elevated temperatures with high atmospheric CO_2 levels. Many works have addressed the effects of individual stressors, such as elevated temperatures or CO_2, on plant metabolism separately as an approach to study the impact of climate change on plants. Thus, it is well known that increasing temperatures up to optimal values leads to higher plant growth rates; however, supraoptimal temperatures dramatically repress plant growth, mainly as a consequence of photosynthesis impairment (Raza et al., 2019). Similarly, increased atmospheric CO_2 levels significantly improve the growth of plants, yet in the long term, the consequent accumulation of carbohydrates inhibits photosynthesis (Thompson et al., 2017). Furthermore, the positive impact of elevated CO_2 may be counteracted by the effect of supraoptimal temperatures (Hatfield et al., 2011). Regarding brassicas, the optimal growth temperature for cauliflower (*B. oleracea* var. *botrytis*)
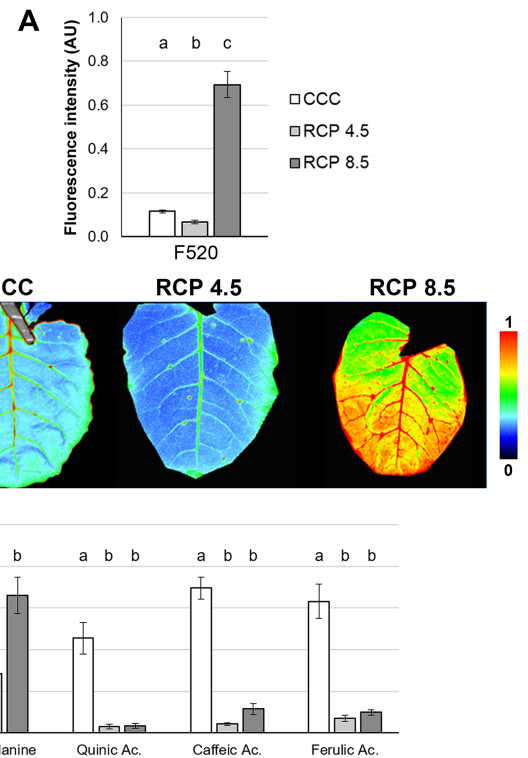

FIGURE 5 Secondary metabolism of broccoli plants (II). **A)** Green fluorescence measured at 520 nm (F520) and emitted by whole leaves. **B)** F520 images of representative whole leaves. A false colour scale is applied. **C)** Relative contents of phenylalanine as precursor of the secondary metabolism, and secondary metabolites of broccoli leaves. Graphs show average normalized values ± standard errors. For graph in **A)** n = 36 whole leaves. For graph in **C)** n = 10 (RCP 8.5) or 12 disks from different leaves (the rest). Lowercase letters indicate statistical differences at $p < 0.05$. Ac: acid.
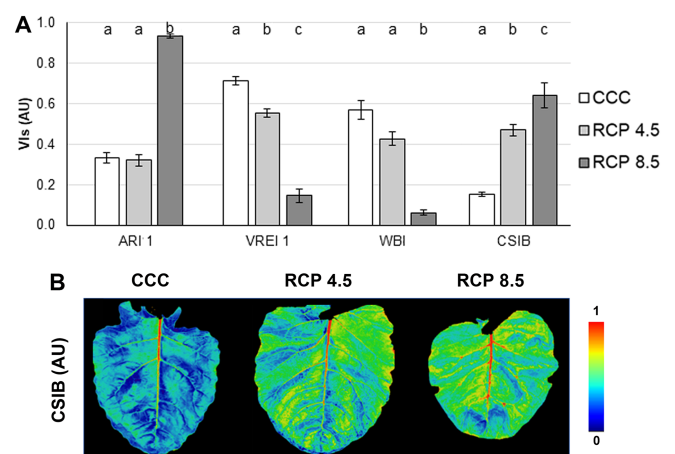

FIGURE 6 Vegetation indices (VIs) providing information on several broccoli plant traits. **A)** Anthocyanin reflectance index 1 (ARI 1), Vogelmann red edge index (VREI 1), water band index (WBI) and climate stress index for brassicas (CSIB). **B)** Representative CSIB images of whole broccoli leaves. Graphs show average normalized values ± standard errors (n = 36 whole leaves). Lowercase letters show statistical differences at $p < 0.05$.

(Kuçi et al., 2012) and broccoli (Rodríguez et al., 2014) was found to be 30°C, whereas, in our experiments, 34 and 37°C were applied for RCP 4.5 and RCP 8.5, respectively. In a similar way, increased CO_2 (up to 740 ppm) also significantly improved the growth of oilseed rape plants (Qaderi et al., 2006, Högy et al., 2010, Kimball, 2016). This value was also exceeded in the case of plants grown at RCP 8.5 (1000 ppm). In this manner, broccoli plants were grown under conditions simulating the forecasted temperatures and CO_2 levels that would, in principle, impose mild and severe stress on the plant.

The extreme projection of climate change, RCP 8.5, produced the greatest alterations in the morphology of broccoli leaves and plant growth. Leaves of plants cultivated at RCP 8.5 were smaller and thicker than those cultivated at CCC or RCP 4.5, as shown by the higher leaf FW per area unit (Figure 1). Indeed, a reduction in leaf thickness has been reported in brassicas upon heat treatment (Rodríguez et al., 2015, Pérez-Bueno et al., 2022). However, high atmospheric CO_2 levels, alone or combined with warm temperatures, cause the opposite effect on canola (*Brassica napus*) (Qaderi et al., 2006). The opposing impact of temperature and CO_2 levels on leaf thickness might explain the contrasting effect of RCP 4.5 and RCP 8.5 conditions on broccoli leaf thickness (Figure 1A). Such disturbances in plant development are common responses to climate change, as reviewed by Gray & Brady (2016). However, plants and leaves of oilseed rape did not undergo drastic developmental changes when cultivated at RCP 4.5 and RCP 8.5 (Pineda & Barón, 2022), suggesting that environmental conditions resulting from climate change would impact differently among Brassica crops.

Climate change will have a great impact on the primary metabolism of broccoli plants. Unaltered values of F_V/F_M (Figure 2A) suggested an effective adaptation of the photosynthetic machinery to each ambient condition by avoiding oxidative damage to the photosystems (Pérez-Bueno et al., 2019). However, the photosynthesis was downregulated under RCP 4.5 and RCP 8.5 conditions since Φ_{PSII} is directly related to the activity of the Calvin cycle and the CO_2 fixation rate (Maxwell & Johnson, 2000). Furthermore, the inhibition of photosynthesis was accompanied by a decrease in the capacity for protective energy dissipation in PSII, measured by NPQ. These results are in accordance with previous results found in brassicas under abiotic stress. For example, Φ_{PSII} decreased in broccoli plants upon severe salt stress (Ali et al., 2022), Similarly, UV-B radiation caused a decrease in the photosynthetic electron transport rate in *Arabidopsis thaliana*, along with a decrease in NPQ (Moon et al., 2011). This inhibition of photochemical and energy-dissipating processes points to a downregulation of PSII. It is worth noting that the lower photosynthesis rates did not appear to be related to senescence since the RCP conditions triggered an increase in Chl levels per fresh weight (Figure 2C). Moreover, alterations in Chl *a/b* ratio would point to changes in the composition of the photosynthetic complexes, their antennae and their relative contents, as reviewed by Pinnola & Bassi (2018).

Ambient CO_2 and temperature would influence the equilibrium between photosynthetic carbon assimilation and the photorespiration process (Gupta, 2020). It is well known that high CO_2 concentrations increase the carboxylase activity of RubisCO over its oxygenase activity. Indeed, this leads to a reduction in the need for photorespiration and, consequently, to a lower production of H_2O_2 by the glycolate oxidase. Another aspect of primary metabolism that would be affected by global warming is nitrogen metabolism. In particular, the capacity for nitrogen fixation, which is dependent on $NADPH^+$ production by the electron transport chain, and could also be affected by the photorespiration rate (Schmidt & Zinkernagel, 2021). However, the CO_2 concentrations used in this work (at least under RCP 4.5, 600 ppm) might not be sufficiently high enough to cause a substantial decrease in the oxygenase activity of RubisCO to residual levels. In fact, Queval et al. (2012) determined a minimum CO_2 concentration of 3000 ppm to ensure a decreased RubisCO oxygenase activity for *A. thaliana*, another C_3 brassica like broccoli.

Plants grown under abiotic stress conditions might prioritize defence over growth. This process is known as the growth-defence trade-offs and plays a pivotal role in plant survival (Figueroa-Macías et al., 2021). This was observed, particularly in plants under RCP 8.5. On one hand, RCP 8.5 plants exhibited growth inhibition (Figure 1B). On the other hand, the increase in the Fru / Glc ratio found under such conditions (Figure 3B) could be indicative of starch degradation (Smeekens et al., 2010). Moreover, the decrease in the Suc to hexoses ratio is often related to an enhancement in the invertase activity (Tauzin & Giardina, 2014). Both results would suggest that plants cultivated under RCP conditions mobilize carbon storage and divert primary carbon flux into the secondary metabolism. Furthermore, the drastic reduction in MI content in plants grown at RCP 4.5 and RCP 8.5 (Figure 3A) might be related to its role as a precursor of stress-related metabolites, such as ascorbic acid, raffinose or galactopinitol, and cell wall polysaccharides (Loewus & Murthy, 2000, Adak et al., 2023), contributing to the adaptation of plants to stress conditions. Indeed, sugars help to tune the secondary metabolism. The regulation of the phenylalanine ammonia-lyase, a key enzyme in the phenylpropanoid pathway, is mediated by variations in the Suc to hexose ratio (Tauzin & Giardina, 2014). In broccoli plants, the phenylalanine content was drastically increased in plants grown under RCP 8.5 (Figure 5C), which is in agreement with previous results (Almuhayawi et al., 2020). The increase in phenylalanine content could be related to an active synthesis of derived secondary metabolites, such as the phenylpropanoids (hydroxycinnamyl alcohols, and hydroxycinnamic acids such as ferulic or coumaric acid), which are precursors of lignin (Vanholme et al., 2019). This assumption would be supported by: i) the increase in F520, indicative of higher lignification and/or accumulation of other phenolics covalently bound to cell walls under RCP 8.5 (Figure 5A, B); ii) the decrease in the PPGs as lignin precursors (Figure 4B).

Future climate conditions would lead to a reduction in leaf transpiration in broccoli plants. A reduction in leaf transpiration can be induced by the activation of stomatal closure and/or by decreasing the stomatal density or size during leaf development. *A. thaliana* decreased leaf transpiration in response to prolonged elevated temperatures (Pérez-Bueno et al., 2022), as well as oilseed rape plants cultivated under high temperature and CO_2 (Qaderi et al., 2006, Pineda & Barón, 2022). Moreover, leaf transpiration is one of the main

factors that drive nutrient uptake by the roots as well as leaf expansion (Pantin et al., 2011). Therefore, low leaf transpiration rates would be linked to an inhibition of broccoli plant growth under RCP 8.5 (Figure 1A).

The phenylpropanoids pathway is usually induced in response to abiotic environmental stresses (salinity, heavy metals, heat or cold, CO_2, etc.), resulting in the accumulation of soluble compounds known for their antioxidant properties. However, the plant response to global warming is poorly understood. Thus, heat treatment resulted in low concentrations of total phenolics in Siberian ginseng plants, or low levels of flavonoids in sugarcane, mung bean and broccoli Calabrais plants (Qaderi et al., 2023). Moreover, high temperatures combined with high atmospheric CO_2 concentrations caused a reduction in the contents of chlorogenic acid (a derivative of quinic acid) and feruloylquinic acid in barley, silver birch and European aspen (Gmižić et al., 2023). In accordance with these results, climate change reduced the amounts of soluble phenolics in broccoli plants (Figures 4B and 5C). A plausible explanation for the low contents of these metabolites could be related to the fact of being precursors of lignin, as already discussed. Another explanation could reside in their antioxidant capacity. Oxidative conditions cause the activation of the antioxidant metabolism, and the oxidation of such compounds would cause a decline in the accumulation levels of their reduced form, quantified by the methods followed in this work, along with the decline in the TAA (Sharma et al., 2019, Vuolo et al., 2019). It is also known that high concentrations of CO_2 can activate redox components, including enzymes of the antioxidant metabolism, thus contributing to a reduction of oxidative stress (Mhamdi & Noctor, 2016). Furthermore, Car and Xanth also exhibit antioxidant properties and are highly accumulated in broccoli plants grown under both RCP conditions (Figure 4A). Indeed, Gmižić et al (2023) described the accumulation of Car in broccoli Calabrais plants (B. oleracea L. convar. botrytis (L.) Alef. var. cymosa Duch.) induced by high temperatures (38°C). Taking together the decrease in TAA and lipid peroxidation levels in plants grown under RCP conditions relative to control plants (Figure 4A), the results suggest an activation of the antioxidant metabolism under RCP conditions. Therefore, under climate change scenarios, both soluble phenolics and Car would contribute to an increase in the antioxidant non-enzymatic pool and consequently to the decrease in the lipid peroxidation levels found in plants grown under RCP 4.5 and RCP 8.5, relative to the controls (Figure 4A).

The decrease in intermediate metabolites of the Krebs cycle, such as citric and malic acid, along with the decrease in Suc and Glc content (Figure 3A, E), would suggest a decrease in the respiration rate. This would be in agreement with slow metabolic activity and the inhibition of plant growth under extreme global warming conditions, compared to CCC. Many works have already shown that the inhibition of plant respiration is related to global warming (Wendering & Nikoloski, 2023). For example, A. thaliana inhibits the Krebs cycle, with decreases in malic and citric acids (Wang et al., 2020). Furthermore, the intermediate metabolites in the Krebs cycle can serve as precursors for different secondary metabolites that would contribute to the adaptation of plants to abiotic stress caused by climate change (Pott et al., 2019).

The evaluation of physiological parameters could be complemented by the analysis of VIs, particularly those that correlate to plant physiological traits, such as pigment contents, fitness or vigour (Roberts et al., 2018), supporting the results about broccoli plant health. For instance, ARI 1 is linked to the amount of anthocyanins present in leaves (Gitelson et al., 2001). These pigments serve as osmotically active solutes and antioxidants for reactive oxygen species in addition to their function as a UV screen (Al-Sammarraie et al., 2020). Broccoli plants increased ARI 1 only when they were cultivated under RCP 8.5, contrasting with oilseed rape plants, which accumulated anthocyanins under both RCP 4.5 and RCP 8.5 (Pineda & Barón, 2022), supporting a differential capacity for climate adaptation among brassicas. Another commonly used VI is VREI 1, which is strongly influenced by Chl concentration, leaf area or water content (Vogelmann et al., 1993). Therefore, lower VREI 1 values of plants cultivated at RCP 4.5 (Figure 6A), and more severely for those grown at RCP 8.5, would indicate lower plant fitness and vigour relative to control plants. This would correlate with the reduced photosynthetic capacity (Figure 2A), as discussed above. Finally, WBI (Figure 6A; Table 2), which negatively correlates with the equivalent water thickness (Watt et al., 2021), would support the increase in thickness of leaves from plants grown at RCP 8.5 (Figure 1B), provided that plants did not undergo water deficit throughout the experiments. Finally, CSIB is a VI developed specifically to detect abiotic stress caused by climate change in oilseed rape plants. Indeed, CSIB showed high values relative to controls the more extreme the climate change scenario imposed on the broccoli plants (Figure 6A, B), confirming that its use might be extended to the detection of abiotic stress in other brassica species.

More research is needed to fully understand the effect of global warming on plant physiology. The rise in CO_2 and temperature related to climate change will have an impact on many aspects of plant metabolism and development. Moreover, the plant responses and the final outcome of climate change on crops would be different depending on whether they are C_3, C_3/C_4 or C_4 species (Gupta, 2020). Improving our knowledge about the capacity for adaptation of particular crops, along with their main defence / adaptive mechanisms to cope with climate change, would be crucial in designing strategies to improve crop tolerance. Future research should address approaches aiming to mitigate the negative impact of global warming on agriculture and food security in the long term.

5 | CONCLUSION

Changes in weather patterns caused by climate change could have a profound impact on broccoli growth and production. Broccoli plants seemed to be able to adjust their primary and secondary metabolism when grown under an intermediate representative concentration pathway (RCP 4.5), in which average temperature would be increased by 3°C. However, deeper changes in primary and secondary metabolism, a higher lignification of cell walls and alterations in plant growth were observed under the most extreme representative concentration

pathway (RCP 8.5). This scenario, with an expected rise in the average temperature of 6°C, would take place if greenhouse gas emissions remained at actual rates. Under these climate conditions, the adaptive capacity of broccoli plants would be exceeded, causing inhibition of plant growth and a decline in plant fitness, likely lowering the crop yield. Understanding the impact of climate change on crop plants would improve our knowledge about how global warming will affect agriculture and food security, which is crucial for the health and well-being of people and the planet. Research focused on combined stressors seems an appropriate approach to fully understand the impact of future climate on plant physiology and crop quality and yield. Consequently, in order to fully understand the impact of future climate on plant physiology, upcoming research should be focused on the combined application of both stressors, as they will occur concomitantly.

AUTHOR CONTRIBUTIONS

MP and MLPB conceived, designed, and conducted the experiments, analysed data, mounted figures and interpreted the results. MB contributed materials, equipment and analysis tools. MP, MLPB and MB wrote the manuscript, and all the authors reviewed it and approved the final version.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.