Research paper

Enhanced photosynthesis, transpiration regulation, water use-efficiency and growth in buckwheat outperforms wheat response to high [CO$_2$], high temperature and drought

Xabier Simón Martínez-Goñi [*], Jon Miranda-Apodaca, Usue Pérez-López

University of the Basque Country (UPV/EHU), Faculty of Science and Technology, Department of Plant Biology and Ecology, Barrio Sarriena, Leioa, Biscay 48940, Spain

A B S T R A C T

The rising atmospheric [CO$_2$] levels will increase global temperature and drought events, threatening wheat (*Triticum aestivum*) production. In recent years, buckwheat (*Fagopyrum esculentum*) has emerged as an alternative crop to wheat under extreme drought. However, it is essential to understand the ability of these species to withstand future climatic conditions where drought and high temperature will occur simultaneously in a high [CO$_2$] environment. Since the mitigating effect of high [CO$_2$] is strongly influenced by the severity of the stress, we compared the response of wheat and buckwheat, differently sensitive to drought, to future climatic scenarios. In wheat, high temperature and high [CO$_2$] passively decreased water potential, as shown by the higher dehydration. Likewise, future drought extremely increased water requirements, causing extreme reductions in the photosynthetic rate and in the quantum yield of PSII, as well as changes in the antioxidant metabolism. Conversely, buckwheat maintained optimal hydration levels, promoted higher photosynthetic rates and increased water-use efficiency under the combination of high [CO$_2$] and high temperature, with and without drought. The improved response of buckwheat was attributed to an enhanced stomatal regulation and water-use efficiency, and resulted in an outperforming growth under future climatic conditions compared to the growth in wheat. Our research highlights the promising potential of buckwheat as an alternative crop to wheat under future climatic scenarios.

1. Introduction

Atmospheric [CO$_2$] has been increasing exponentially over the past decades and it is expected to keep increasing by the end of the century (www.co2.earth). According to the last reports of the Intergovernmental Panel on Climate Change (IPCC), [CO$_2$] will continue to rise from the current approximate 400–700 ppm by 2070 (The Core Writing Team IPCC, 2021, 2015). As a consequence, greenhouse effect will be enhanced, leading to a likely increase in global temperatures of 3 °C (The Core Writing Team IPCC, 2021, 2015). High temperatures will in turn increase water evaporation, intensifying water scarcity in different regions of the planet. Thus, future crops will be facing drought episodes in a high [CO$_2$] and high temperature environment (The Core Writing Team IPCC, 2021, 2015).

High temperature and drought have been reported to negatively affect conventional crops as wheat (*Triticum aestivum*). In fact, in a review paper by Farooq et al., (2009) it was discussed how drought induces stomatal closure and transpiration decrease in order to prevent water loss, leading to the inhibition of net photosynthetic [CO$_2$] assimilation and damages of PSII when maintained for long periods. In contrast, under high temperature stomatal aperture and transpiration rates are initially stimulated as a short-term adaptive response to mitigate leaf overheating (Mathur et al., 2014). However, over the long-term stomata are also closed due to the increased water demand, causing limitations in the net photosynthetic [CO$_2$] assimilation (Mathur et al., 2014; Prasad et al., 2008). Consequently, high temperature and drought cause an overproduction of reactive oxygen species (ROS), damaging even more the photosynthetic apparatus and provoking an impairment of photochemical reactions (Huang et al., 2001; Reddy et al., 2004). In addition, high temperature promotes photorespiration and respiration, limiting the net photosynthetic [CO$_2$] assimilation and carbon assimilation in wheat (Dusenge et al., 2019; Ku and Edwards, 1977a, 1977b). Consequently, high temperature and drought cause limitations in growth, which result in yield losses in wheat (Mahrookashani et al.,

2017; Prasad et al., 2011).

On the contrary, high [CO_2] has been shown to alleviate the negative impact of high temperature or drought in crops (Ainsworth and Long, 2021; Lee, 2011; Martínez-Goñi et al., 2023b). This alleviating effect could primarily be attributed to the increased availability of [CO_2], which enhances carbon-fixation processes, increasing net [CO_2] assimilation and thus, stimulating biomass production (Dias de Oliveira et al., 2015; Drake et al., 1997; Kimball, 2016). Additionally, high [CO_2] alone has been shown to decrease stomatal conductance (*gs*) and increase transpiration regulation in wheat, leading to an enhanced water use-efficiency (*WUE*) and improved water status (Li et al., 2019; O'Leary et al., 2015; Wall et al., 2006). However, these responses are dependent on stress severity. In fact, it has been reported that the combination of high [CO_2] with temperatures above 2 °C to ambient, increased biomass and grain yield in two genotypes of wheat due to higher photosynthetic rates and greater leaf area. However, temperatures higher than 2 °C to ambient restricted the positive effects due to a less effective stomatal responsiveness to high [CO_2] in wheat (Dias de Oliveira et al., 2013). Considering that drought also alters *gs* and instantaneous transpiration (*E*), and therefore has the potential to change the established stomatal response under high [CO_2] and high temperature, the study of the combined impact of these three environmental variables on wheat becomes of great concern.

Studies have shown that high [CO_2] mitigates the detrimental effects of the combination of high temperature and drought, although a general mechanism cannot be concluded. Zinta et al. (2014) found that high [CO_2] alleviates the detrimental effects of high temperature and drought in *Arabidopsis thaliana* by increasing the synthesis of antioxidant metabolites as ascorbate (*AsA*). Nonetheless, in some grassland species the mitigation was observed due to reduced rates of photorespiration, which contributed to a decrease in oxidative damage (AbdElgawad et al., 2015). In the same manner, high [CO_2] has also been reported to promote photosynthesis and increase carbon assimilation in summer rape, resulting in a better maintenance of growth under high temperature and drought (Kacienė et al., 2019). Likewise, high [CO_2] has also been reported to induce reductions in *gs* and *E* in *Brassica napus*, resulting in an enhancement in the water-use efficiency, contributing an improved water status within the plant (Dikšaitytė et al., 2019). As it is demonstrated, the underlying mechanisms are not fully understood, since not all plant species demonstrate similar response under the triple interaction (Nguyen et al., 2017). In this research, we delve deeper into wheat, given its importance as staple food (Garg et al., 2021). When wheat is grown under terminal drought, high [CO_2] and temperatures 2 °C above ambient, the negative effects of drought on biomass and yield are reduced (Dias de Oliveira et al., 2013). Not only that, but the response was variety-specific, with one variety exhibiting higher photosynthetic rates while in the other the tillering capacity was higher (Dias de Oliveira et al., 2013). However, those studies did not consider other significant mechanisms, such as root biomass and antioxidant capacity, among others. Besides, when ambient temperature was increased in more than 2 °C in a high [CO_2] environment, *gs* and *E* were not decreased, causing higher loss of water through transpiration that led to a greater impact of drought and subsequent limitation in aboveground biomass (Dias de Oliveira et al., 2013). Therefore, the extent to which high [CO_2] ameliorates the impact of high temperature in wheat is dependent on the experienced stress level, and this effect may decrease beyond the 2 °C increase threshold. Hence, the projected 3 °C rise in global temperatures is expected to exceed the aforementioned stress-threshold of wheat (Dias de Oliveira et al., 2013; The Core Writing Team IPCC, 2021). As a result, it is reasonable to hypothesise that the beneficial impact of high [CO_2] could be even more reduced when it is combined with both high temperatures and drought. Consequently, conducting further research is crucial to develop a comprehensive understanding of how wheat responds to the complex interactions between high [CO_2], high temperatures and drought.

Given the global reliance on the conventional crop wheat, its yield

losses threatens the ability of millions to access sufficient food (Ainsworth and Long, 2021; FAOSTAT, 2021). Therefore, there is an urgent need to find alternative crops capable of withstanding future environmental conditions. Alternative crops are those that are not commonly grown around the world when compared with conventional crops, and are cultivated with the goal of diversifying agricultural production (Rodríguez et al., 2020). Due to their ability to grow in harsh environments better than conventional crops, alternative crops have been gaining much attention in the last years (Cheng, 2018). Buckwheat (*Fagopyrum esculentum*) is an underutilized C_3 pseudo-cereal which has demonstrated superior resilience compared to wheat when exposed to drought. Specifically, while the net photosynthetic [CO_2] assimilation (*A*) of wheat was already limited under mild drought conditions, buckwheat exhibited greater leaf transpiration regulation, preservation *A*, improved *WUE* and withstanding growth over compared wheat when exposed to extreme drought (Martínez-Goñi et al., 2023a). While previous research have explored the individual responses of buckwheat to drought and high temperature (Aubert et al., 2021, 2020; Cawoy et al., 2006; Martínez-Goñi et al., 2023a), its capacity to withstand their combined effect under future [CO_2] levels remains unexplored. Thus, to test the suitability of buckwheat as an alternative crop to wheat for the future, we have characterised the response of the conventional crop wheat and the alternative crop buckwheat to the simultaneous interaction of the future climatic stresses, including the combination of high [CO_2], high temperature and drought. We hypothesise that 1) the improved regulation of leaf transpiration observed in buckwheat under water-demanding conditions will persist when exposed to high [CO_2] and a 3 °C increase, thereby exhibiting a positive response, 2) when subjected to the combination of high [CO_2], high temperature and drought, wheat will be more severely damaged than under the combination of high [CO_2] and high temperature and 3) buckwheat will exhibit enhanced resilience under the combination of high [CO_2], high temperature and drought. As far as we know, this is the first time that a research reports the interplay among water relations, photosynthetic parameters, antioxidant metabolism and growth related parameters of buckwheat to the combination of two and three stresses of the future climate change conditions.

2. Materials and methods

2.1. Plant material and growth conditions

Seeds of *Tritium aestivum* var. Florence Aurora (wheat) and *Fagopyrum esculentum* var. Kora (buckwheat) were acquired from the Basque Institute for Agricultural Research and Development (NEIKER, Basque Country, Spain). Plants were sown in 3 L pots filled with a mixture of perlite:vermiculite (3:1 v/v). Two plants per pot were grown, and the experiment was designed to ensure a minimum of 10 plants per species and treatment.

Plants were grown in a Conviron PGR15 (Conviron, North Dakota, USA) growth chamber under two controlled environments: 400 ppm [CO_2] and 24/18 °C for day/night (ambient [CO_2] and temperature, ACT), and 700 ppm [CO_2] and 27/21 °C for day/night (future [CO_2] and temperature, ECT). The photoperiod was maintained at 16 hours and 400 photosynthetically active radiation (PAR), with humidity levels at 60/80% for day/night. Pots were watered (well-water treatment, W) to field capacity (FC) three times a week using a complete Hoagland solution (Hoagland and Arnon, 1938), and rotated to prevent intra-chamber environmental gradients. 14 days after sowing (DAS), half of the plants were subjected to drought by withholding water until the substrate reached 20% ± 5 FC, which was maintained for 1-week (drought treatment, D). Therefore, four treatments were applied: ambient well-watered conditions (ACTW), ambient drought conditions (ACTD), future well-watered conditions (ECTW) and future drought conditions (ECTD).

All the *in vivo* measurements and plant material harvesting was

performed at the end of the drought treatments, which were: under ACTD, on average after 37 DAS and 29 DAS for wheat and buckwheat, respectively; and under ECTD, on average after 39 DAS and 27 DAS for wheat and buckwheat, respectively. It was ensured that for every pot harvested under D, there was one harvested under W. At the time of harvesting, wheat plants were ending the booting stage, whereas buckwheat plants were at flowering.

2.2. Soil and water parameters

Soil and plant water parameters were analysed as described in Martínez-Goñi et al. (2023a). Briefly, cumulative transpiration (*CuTr*) of all the pots per treatment was determined by weighing pots three times per week before and after watering and subtracting water loss by substrate evaporation. The water loss by substrate evaporation was measured using 3 pots filled with substrate without plants, and had average values of 107 g day^{-1}, 48 g day^{-1}, 146 g day^{-1} and 54 g day^{-1} under ACTW, ACTD, ECTW and ECTD, respectively. Soil relative water content (*SRWC*) was calculated as follows: $SRWC = 100 \, [(SFW - SDW) / (SFWi - SDW)]$, where *SFW* is the substrate fresh-weight, *SDW* is the substrate dry-weight and *SFWi* is the initial substrate fresh-weight, respectively.

Leaf water potential (Ψ_w) was measured six hours after dawn using the Scholander pressure-equilibration technique (Scholander et al., 1965). However, due to its high Ψ_w value, Ψ_w in buckwheat could not be quantified by the pressure chamber method (Delpérée et al., 2003; Martínez-Goñi et al., 2023a). The leaf osmotic potential (Ψ_o) was determined by analysing the freezing point of the sap of leaf segments using an OSMOMAT 030 cryoscopic osmometer (Gonotec GMBH, Berlin, Germany) and calculated as $\Psi_o = M \times T \times R$, where M is the concentration (osmol), T is the temperature of the sample (298 K) and R is the molar gas constant (0.00832 L MPa K^{-1} mol^{-1}). Pressure potential (Ψ_p) was calculated as the difference between Ψ_w and Ψ_o. The osmotic potential at full turgor (Ψ_o^{100}) was determined similarly to Ψ_o. This was done by cutting leaves per species and treatment, and soaking them in deionized water at 4 °C in darkness for 24 h.

Leaf relative water content (*LRWC*) was calculated by $LRWC = 100 \times (FW - DW)/(TW - FW)$, with *FW* representing the leaf fresh-weight, *DW* representing the leaf dry-weight and *TW* representing the turgid weight. Dehydration (*DH*) was determined as the difference between Ψ_o and Ψ_o^{100}. Osmotic adjustment (*OA*) was calculated as the difference in the Ψ_o^{100} between D and the W. Cell wall elasticity (ε) was calculated as $\varepsilon = (\Psi_p^{100} - \Psi_p)/(100 - LRWC) \times 100$.

2.3. Gas exchange and photochemical parameters

Gas exchange parameters were measured in fully developed leaves using a Li-Cor 6400 instrument (Li-Cor Inc., Lincoln, NE, USA). Each plant was measured after reaching steady-state conditions, which typically occurred at 7 min. This allowed sufficient time for the stomatal conductance (*gs*) to stabilize and ensured consistency in the data collection. Airflow in the chamber was 250 μmol s^{-1}. In wheat, measurements were taken on the leaf preceding the Flag Leaf, while in buckwheat, a fully developed intermediate leaf was used for the measurement. The temperature of the cuvette was kept at 24 °C for ACT and 27 °C for ECT, with a relative humidity of 60%. Measurements were made three hours after dawn under a photosynthetic photon flux density (PPFD) of 400 μmol m^{-2} s^{-1} provided by a red/blue LED light source with a 10% blue light (model Li 6400–40, Li-Cor Inc.). Average VPD values were 1.58 kPa and 1.68 kPa under ACT and ECT, respectively. *gs*, net photosynthetic [CO$_2$] assimilation (*A*) and instantaneous transpiration (*E*) were calculated according to von Caemmerer and Farquhar (1981).

The quantum yield of PSII (Φ_{PSII}) was determined using an integrated leaf fluorescence chamber (Li-6400–40, Li-Cor Inc.), and was calculated as $\Phi_{PSII} = (Fm' - Fs)/Fm'$, where *Fs* represents the variable fluorescence at steady state and *Fm'* the maximum light-adapted fluorescence (Schindler and Lichtenthaler, 1996). Electron transport rate (*ETR*) was calculated as $ETR = \Phi_{PSII} \times PPFD \times 0.85 \times 0.5$, where PPFD refers to the photosynthetic photon flux density. The ratio of electron transport rate to net photosynthetic [CO$_2$] assimilation (*ETR/A*) was calculated as the ratio between *ETR* and *A*.

2.4. Antioxidant parameters

Superoxide dismutase (*SOD*), catalase (*CAT*), glutathione reductase (*GR*) and ascorbate peroxidase (*APX*) were determined as described by Pérez-López et al. (2009), with little modifications.

Shortly explained, *SOD*, *CAT* and *GR* were extracted from the samples using a buffer composed by 50 mM Tris-HCl (pH 7.8), 0.1 mM ethylenediaminetetraacetic acid (EDTA), 0.2% Triton X-100, 0.1 mM phenylmethylsulfonyl fluoride (PMSF) and 2 mM dithiotreitol. The supernatant of the samples was filtered through Shepadex G-25 columns equilibrated with 50 mM Tris–HCl (pH 7.8), 0.1 mM EDTA and 0.2% Triton X-100. *CAT* was measured at 240 nm as per the method described by (Aebi, 1984), *GR* activity was measured at 340 nm according to Edwards et al. (1990), and the activity of *SOD* was measured at 550 nm as described by McCord and Fridovich (1969). *APX* was extracted in a buffer of 50 mM KH$_2$PO$_4$ and K$_2$HPO$_4$ (pH 7.8), containing 0.1 mM EDTA, 0.2% Triton X-100, 5 mM cysteine, 2 mM ascorbate (*AsA*) and 0.1 mM PMSF. *APX* activity was measured at 290 nm according to Hossain and Asada (1984). All enzyme activities were expressed on a protein basis, which was measured using the Bradford method (1976).

Total Glutathione (*GSH + GSSG*) and *Total Ascorbate* (*AsA + DHA*) were measured as described by Pérez-López et al. (2010) with modifications. Briefly, extraction from the samples was done using an extraction buffer composed of 3% HCl and 1 mM EDTA. *Total Glutathione* supernatant was neutralized using a mixture of 1 M succinate, 0.5 M potassium hydroxide (KOH) and 250 mM Tris buffer (pH 7) containing 6.3 mM EDTA and 0.32 mM NADPH. For the *Total Glutathione* measurement, 6 mM 5,5′-ditiobis (2-nitrobenzoic) acid (DTNB) and 2.1 U ml^{-1} *GR* were added, as explained by Griffith (1980). *Total Glutathione* was measured at 412 nm. Reduced ascorbate (*AsA*) was quantified by adding 100 U ml^{-1} ascorbate oxidase and measuring the reduction in absorbance at 265 nm. Oxidized ascorbate (dehydroascorbate, *DHA*) was determined by adding 300 mM dithiotreitol and measuring the increase in absorbance at 265 nm.

2.5. Growth parameters

For each treatment, specific plants of every plant species were harvested and divided into leaves, stems, and roots. Plant organs were ovendried for 72 h to determine their dry-weight (*DW*), and water use efficiency (*WUE*) was calculated by dividing the *Total DW* by the *CuTr* of each specific plant. The sum of *Leaf DW* and *Stem DW* (*Shoot*) was divided by *Root DW* to determine *Shoot/Root* ratio. Leaf area (*LA*) measurements were made as explained in Martínez-Goñi et al. (2023a). Briefly explained, photos of the harvested fresh leaves were used to calculate *LA* with the open-source software Fiji (Fiji Is Just ImageJ; Schindelin et al., 2012).

Relative growth rate (*RGR*) was calculated as $RGR = \ln(DW_2/DW_1)/(t_2 - t_1)$ where DW_1 and DW_2 refer to the total DW of the plant at times t_1 and t_2, respectively, and t_1 and t_2 refer to 14 DAS (start of *D*) and the harvesting DAS. Leaf area ratio (*LAR*) was calculated as $LAR = (A_2 - A_1)/(DW_2 - DW_1) \times \ln(DW_2/DW_1)/\ln(A_2/A_1)$ where A_1 and A_2 refer to the leaf area (*LA*) at t_1 and t_2, respectively. Net assimilation rate (*NAR*) was determined as $NAR = (DW_2 - DW_1)/(A_2 - A_1) \times \ln(A_2/A_1)/(t_2 - t_1)$. Specific leaf area (*SLA*) was calculated as $SLA = (A_2 - A_1)/(LDW_2 - LDW_1) \times \ln(LDW_2/LDW_1)/\ln(A_2/A_1)$, where LDW_1 and LDW_2 refer to the *Leaf DW* at times t_1 and t_2. Leaf weight ratio (*LWR*) was determined as $LWR = (LDW_2 - LDW_1)/(DW_2 - DW_1) \times \ln(DW_2/DW_1)/\ln(LDW_2/LDW_1)$.

2.6. Stomatal parameters

Leaf imprints from both the adaxial and abaxial surfaces were created as described by (Casado-García et al., 2020). Three photos of the adaxial (*AD*) and three photos of the abaxial (*AB*) surfaces per species and treatment were taken using a Nikon ECLIPSE 50i fluorescence microscope (Nikon corporation, Japan) and a Leica DFC 420 C camera (Leica Microsystems, Germany).

Stomatal measurements were taken with the LabelStoma tool (Casado-García et al., 2020), or were derived from data generated in the LabelStoma tool. In brief, stomatal density (*SD*), length (*L*) and width (*W*) were determined by LabelStoma. Stomatal distribution (*R*) was calculated as $R = SD_{AD}/(SD_{AD} + SD_{AB})$. Stomatal size (*S*) for each surface was defined as $S = L \times W$.

2.7. Statistical analysis

All figures of were made using GraphPad Prism software version 8.2.1 (GraphPad Software, San Diego, CA USA, www.graphpad.com). The statistical analysis was performed using SPSS software version 28.0.1.1 for Windows (IBM Corp, Armonk, NY USA). Our experimental unit was each individual plant. Two-way ANOVA was used to determine the effect of environmental treatment (ACT and ECT), water treatment (W and D) and their interaction in each species (Table 1). Duncan's multiple range test was used to compare the means, and $P < 0.05$ values were considered to be statistically significant. Outliers for each parameter were identified using Grubbs's test. Levene's test was used to assess the homogeneity of variance, while Kolmogorov-Smirnov test was used to evaluate the normality of data distribution. In cases where normality was not met, data transformation was performed prior to ANOVA testing.

3. Results

3.1. Water parameters

In wheat, Ψ_w was affected by the water and environmental treatments, while Ψ_o was only affected by the water treatment and Ψ_p was not affected by any treatment (Table 1 and Table 2). Specifically, Ψ_w was significantly reduced by 68% when exposed to ACTD and ECTW, while ECTD decreased Ψ_w by 114% compared to ACTW (Table 2). Ψ_o, however, was only affected by ECTD, as it was significantly decreased from an average value of -1.82 MPa to -2.32 MPa (Table 2). Similarly, ε was only decreased under ECTD, were it reached values 47% lower than under ACTW. As previously explained, Ψ_w of buckwheat could not be measured (see Materials and Methods). Thus, Ψ_w, Ψ_p and ε could not be calculated for this species. As opposed to wheat, in buckwheat, Ψ_o remained unchanged regardless of the treatment (Table 1 and Table 2).

Wheat had significantly higher *LRWC* values in comparison to buckwheat for all the treatments, especially under ECT (Tables 1 and 2). Intriguingly, we found no variation in *LRWC* values for wheat. Unlike *LRWC*, *DH* levels in wheat progressively increased from nearly 0 under ACTW to 0.249, 0.315 and 0.815 under ACTD, ECTW and ECTD, respectively (Table 2). Furthermore, *OA* was higher in wheat under ECT compared to ACT. In buckwheat, however, *DH* and *OA* levels remained nearly constant across all treatments.

Overall, we found *CuTr* in wheat to be affected by both treatments, while in buckwheat it was only affected by water treatment (Fig. 1 and Table 1). When exposed to ACTD, *CuTr* was significantly decreased in both species. Also, while in wheat ECTW alone significantly decreased *CuTr*, in buckwheat ECTW values were similar to those observed for ACTW.

On average, wheat and buckwheat reached 20% *SRWC* at 37 DAS and 29 DAS under ACTD and 39 DAS and 27 DAS under ECTD, respectively (Fig. 2).

3.2. Stomata data

When analysing *SD*, we found the lower *Total SD* values of wheat to be influenced by the water treatment (Fig. 3 and Table 1). Upon exposure to ACTD, *Total SD* was increased by 42% in wheat, primarily due to the increase in SD_{AD}. On the contrary, in buckwheat *Total SD* was influenced by water treatment and by the interaction among treatments (Table 1), and was significantly increased by 108%, 39% and 59% under ACTD, ECTW and ECTD in comparison to *Total SD* under ACTW (Fig. 3).

Regardless of the treatment, *S* remained unchanged in wheat, while buckwheat showed variations across treatments (Table 1 and Table 3). In fact, the stomatal size in buckwheat was decreased by 33%, 14% and 21% upon exposure to ACTD, ECTW and ECTD, respectively (Table 3). *R* had consistent values across treatments, with wheat showing steady values close to 0.50 and buckwheat close to 0.28 (Tables 1 and 3).

3.3. Gas exchange parameters

Overall, *gs* and *E* were influenced by the water and the environmental treatment in both species, but not by their interaction (Fig. 4A-B and Table 1). Also, wheat showed higher *gs* and *E* regardless of the treatment. Under exposure to ACTD, ECTW and ECTD *gs* and *E* values of wheat were approximately 56%, 26% and 69% lower than under ACTW, respectively (Fig. 4A-B). In buckwheat, *gs* and *E* were 60% lower under

Table 1

Two-way ANOVA for water treatment (W), environmental treatment (CO2*T) and their interaction for all the analysed variables in wheat and buckwheat. Results are represented as ns (non-significant), * (P < 0.05), ** (P < 0.01), *** (P < 0.001) and **** (P < 0.0001).

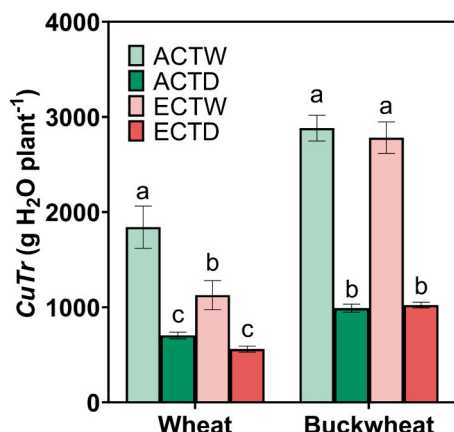|	Wheat			Buckwheat		
	W	CO$_2$*T	W × CO$_2$*T	W	CO$_2$*T	W × CO$_2$*T
Ψ_w	****	****	ns	-	-	-
Ψ_o	***	ns	ns	ns	ns	ns
Ψ_p	ns	ns	ns	-	-	-
LRWC	ns	ns	ns	ns	****	ns
DH	***	****	ns	ns	ns	ns
CuTr	****	**	ns	****	ns	ns
SD$_{AB}$	ns	ns	ns	****	ns	*
SD$_{AD}$	*	ns	*	****	ns	*
Total SD	*	ns	ns	****	ns	***
S$_{AB}$	ns	ns	ns	****	ns	**
S$_{AD}$	*	ns	ns	****	ns	***
R	ns	ns	ns	ns	ns	ns
gs	****	**	ns	*	****	ns
E	****	***	ns	*	****	ns
A	****	ns	***	****	****	**
Φ_{PSII}	****	****	***	****	**	ns
ETR/A	****	*	ns	ns	****	ns
APX activity	****	ns	ns	**	***	**
SOD activity	***	ns	ns	ns	*	ns
CAT activity	ns	ns	ns	ns	ns	ns
GR activity	ns	**	*	**	****	ns
Total Glutathione	ns	**	ns	ns	ns	ns
AsA	ns	ns	ns	ns	ns	*
DHA	ns	*	ns	*	ns	ns
Total Ascorbate	ns	ns	ns	ns	ns	ns
Leaf DW	****	*	ns	****	ns	ns
Stem DW	****	**	ns	****	ns	ns
Root DW	**	*	ns	*	*	ns
Total DW	****	*	ns	****	ns	ns
LA	****	**	*	****	ns	ns
Shoot/Root	****	ns	ns	****	*	ns
RGR	****	****	ns	****	ns	ns
NAR	ns	****	ns	ns	****	ns
LAR	***	ns	ns	****	****	ns
SLA	ns	ns	ns	****	***	ns
LWR	***	ns	ns	ns	***	ns
WUE	ns	**	ns	ns	**	ns

Table 2

Effect of ambient well-water (ACTW), ambient drought (ACTD), future well-water (ECTW) and future drought (ECTD) treatments in wheat and buckwheat for water potential (Ψ_w), osmotic potential (Ψ_o), pressure potential (Ψ_p), cell wall elasticity (ε), leaf relative water content (*LRWC*), dehydration (*DH*) and osmotic adjustment (*OA*). For each species and treatment mean ± S.E. of at least 8 replicates is showed. Different letters are used to represent values significantly different between treatments in each species (P < 0.05).

Species	Treatment	Ψ_w (MPa)	Ψ_o (MPa)	Ψ_p (MPa)	ε (MPa)	LRWC (%)	DH (MPa)	OA (MPa)
Wheat	ACTW	-0.651 ± 0.062[a]	-1.751 ± 0.053[a]	1.120 ± 0.1[a]	10.133	92.78 ± 1.11[a]	-0.081 ± 0.064[c]	-0.0775
	ACTD	-1.098 ± 0.076[b]	-1.992 ± 0.074[a]	0.894 ± 0.108[a]	10.559	91.95 ± 1.23[a]	0.249 ± 0.083[b]	
	ECTW	-1.086 ± 0.085[b]	-1.700 ± 0.086[a]	0.913 ± 0.154[a]	9.263	91.68 ± 0.77[a]	0.315 ± 0.102[b]	0.165
	ECTD	-1.393 ± 0.079[c]	-2.312 ± 0.177[b]	1.114 ± 0.181[a]	5.366	90.37 ± 0.92[a]	0.815 ± 0.146[a]	
Buckwheat	ACTW	-	-1.179 ± 0.093[a]	-	-	81.12 ± 1.74[a]	0.200 ± 0.043[a]	0.099
	ACTD	-	-1.270 ± 0.061[a]	-	-	77.92 ± 1.62[a]	0.192 ± 0.094[a]	
	ECTW	-	-1.122 ± 0.138[a]	-	-	68.86 ± 2.56[b]	0.180 ± 0.090[a]	-0.1775
	ECTD	-	-0.995 ± 0.111[a]	-	-	71.91 ± 2.37[b]	0.230 ± 0.089[a]	

Fig. 1. Effect of ambient well-water (ACTW), ambient drought (ACTD), future well-water (ECTW) and future drought (ECTD) treatments in wheat and buckwheat in cumulative transpiration (*CuTr*). The applied treatments are represented as ACTW (light green bars), ACTD (dark green bars), ECTW (light red bars) and ECTD (dark red bars). Each bar represents mean ± standard error (S.E.) of at least 7 replicates. Different letters are used to represent values significantly different between treatments in each species (*P* < 0.05).
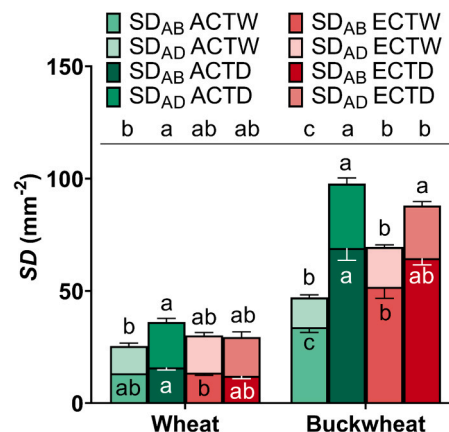

Fig. 2. Effect of ACTD and ECTD treatments in soil relative water content (*SRWC*) over time in wheat and buckwheat. Solid lines represent ACTD treatments, while dashed lines represent ECTD treatments. Blue circles and green triangles are used to represent results for wheat and buckwheat, respectively. Black line is used to represent control treatment (ACTW and ECTW). Each point represents the mean ± S.E. of 9 replicates. Treatments are explained in Fig. 1. DAS means days after sowing.

ECT in comparison to ACT (Fig. 4A-B). Interestingly, *gs* and *E* values were not affected by D treatments in buckwheat, as ACTD and ECTD values were statistically similar to those observed for ACTW and ECTW (Fig. 4A-B).

Fig. 3. Effect of ACTW, ACTD, ECTW and ECTD treatments in the stomatal density (*SD*) of wheat and buckwheat. Green and red colour palettes are used to represent ACT and ECT treatments, respectively. Within these palettes, variations in colour intensity denote specific aspects: pale shades represent adaxial stomata for ACTW and ECTW, while light intensities represent abaxial stomata for ACTW and ECTW. On the other hand, vivid intensities represent adaxial stomata for ACTD and ECTD, while dark intensities represent abaxial stomata for ACTD and ECTD. Each bar represents mean ± S.E. of at least 6 replicates. Treatments and statistical analysis are explained in Fig. 1.
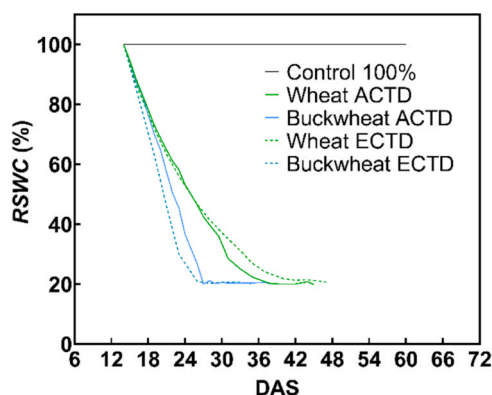
Table 3

Effect of ACTW, ACTD, ECTW and ECTD in stomatal size (*S*) for adaxial (S_{AD}) and abaxial (S_{AB}) surfaces and stomatal distribution (*R*). For each species and treatment mean ± S.E. of at least 4 replicates is showed. Different letters are used to represent values significantly different between treatments in each species (P < 0.05).

Species	Treatment	$S_{AD}(\mu m^{-2})$	$S_{AB}(\mu m^{-2})$	R
Wheat	ACTW	6.130 ± 0.300[a]	5.427 ± 0.162[a]	0.467 ± 0.035[b]
	ACTD	5.084 ± 0.155[a]	4.768 ± 0.126[a]	0.554 ± 0.026[a]
	ECTW	5.833 ± 0.403[a]	5.742 ± 0.422[a]	0.549 ± 0.018[a]
	ECTD	5.363 ± 0.382[a]	5.332 ± 0.381[a]	0.57 ± 0.022[a]
Buckwheat	ACTW	2.315 ± 0.077[a]	1.794 ± 0.081[a]	0.294 ± 0.018[a]
	ACTD	1.438 ± 0.063[c]	1.315 ± 0.085[c]	0.297 ± 0.023[a]
	ECTW	1.978 ± 0.063[b]	1.587 ± 0.039[b]	0.262 ± 0.014[a]
	ECTD	1.799 ± 0.092[b]	1.466 ± 0.048[bc]	0.265 ± 0.014[a]

Although wheat exhibited higher levels of *A* under ACT conditions compared to buckwheat, *A* values in wheat under ACTD were significantly lower compared to ACTW (Fig. 4 C). In contrast, *A* in buckwheat was not influenced by ACTD (Fig. 4 C). Furthermore, while ECTW had no impact on *A* in wheat and ECTD significantly decreased it by 53% compared to ACTW (Fig. 4 C), buckwheat showed a remarkable response of *A* under ECT treatments (Fig. 4 C and Table 1). As a matter of fact, *A* was 57% higher under ECTW in buckwheat in comparison to ACTW, and even though under ECTD it was decreased, it remained 25%
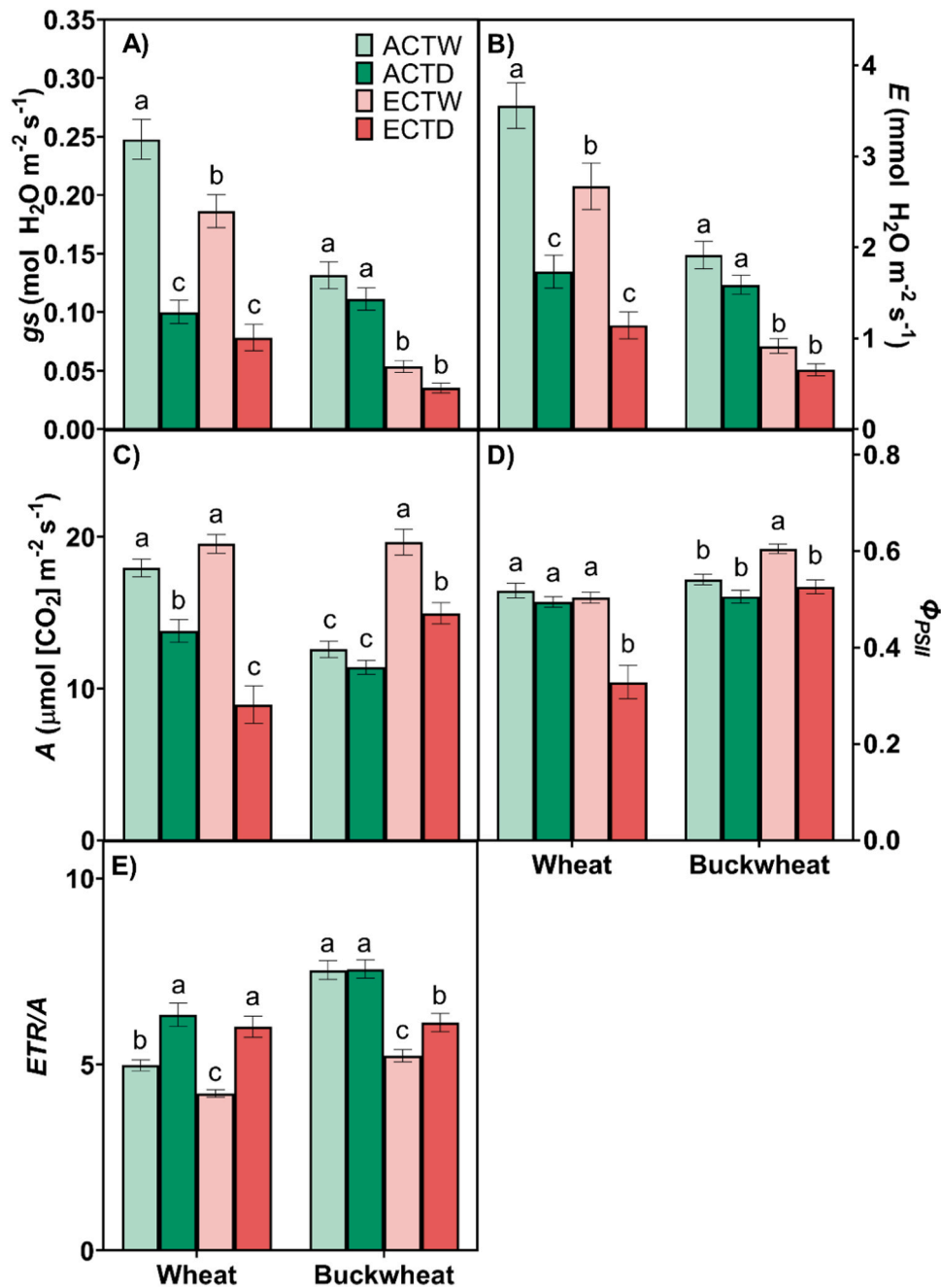
Fig. 4. Effect of ACTW, ACTD, ECTW and ECTD treatments in wheat and buckwheat in **A)** stomatal conductance (*gs*), **B)** transpiration rate (*E*), **C)** net photosynthetic [CO_2] assimilation (*A*), **D)** quantum yield of PSII (*Φ_{PSII}*) and **E)** the ratio of electron transport rate to net CO_2 photosynthetic assimilation (*ETR/A*). Each bar represents mean $\pm$ S.E. of at least 15 replicates. Treatments and statistical analysis are explained in Fig. 1.

higher than under ACT treatments (Fig. 4 C).

Both wheat and buckwheat showed steady values of *Φ_{PSII}* at 0.515 across almost all treatments (Fig. 4D and Table 1). However, under ECTD, *Φ_{PSII}* of wheat was 35% lower than under ACT, while in buckwheat *Φ_{PSII}* under ECTW it was 16% higher than under ACT (Fig. 4D).

ETR/A in wheat was primarily influenced by the water treatment, whereas in buckwheat the environmental treatment was the affecting treatment (Fig. 4E and Table 1). Under ACTW, wheat showed an *ETR/A* value of 4.98, which was increased by 28% when subjected to ACTD (Fig. 4E). Under ECTW, it was decreased by 16%, while it reached similar values to those under ACTD when it was exposed to ECTD (Fig. 4E). Conversely, in buckwheat *ETR/A* had a value of 7.54 under ACTW, which remained similar under ACTD and was 31% and 19%

lower under ECTW and ECTD in compared to ACTW (Fig. 4E).

3.4. Antioxidant parameters

In regard to the antioxidant enzymes, we found *SOD activity* to be affected by water treatment in wheat and the environment in buckwheat (Fig. 5 A and Table 1). Upon exposure to ACTD and ECTD, *SOD activity* was increased in wheat by 60% and 114% compared to ACTW (Fig. 5 A). In contrast, we found almost no variation in *SOD activity* on buckwheat. Similarly, *APX activity* was influenced by water treatment in wheat, and by both treatments and their interaction in buckwheat (Fig. 5B and Table 1). When exposed to ACTD and ECTD, *APX activity* was increased in wheat by 65% and 38% compared to ACTW (Fig. 5B). However, in
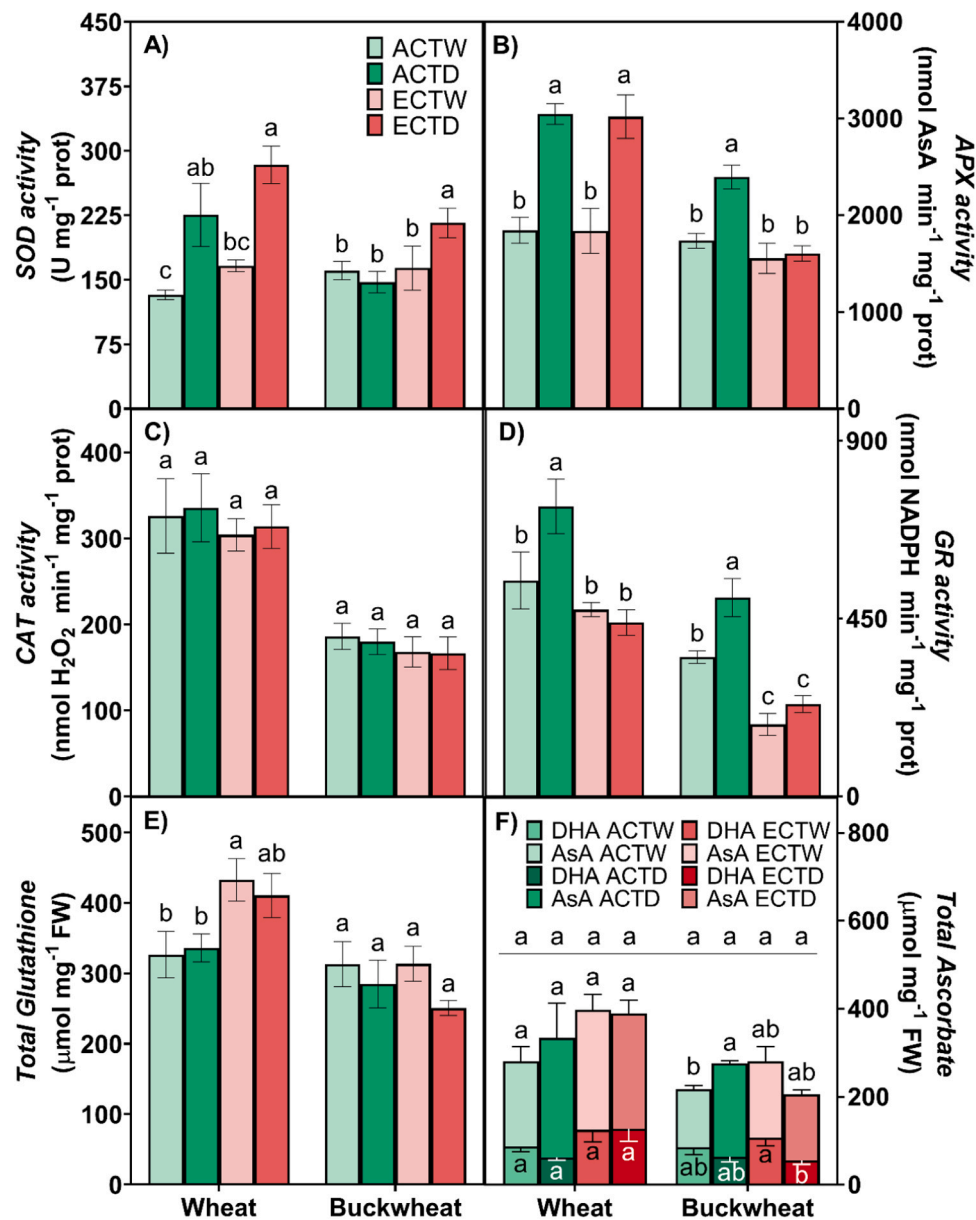
Fig. 5. Effect of ACTW, ACTD, ECTW and ECTD treatments in wheat and buckwheat in **A)** *SOD activity*, **B)** *APX activity*, **C)** *CAT activity*, **D)** *GR activity*, **E)** *Total Glutathione* and **F)** *Total Ascorbate*. Each bar represents mean ± S.E. of at least 4 replicates. Treatments and statistical analysis are explained in Fig. 1.

buckwheat we found an increase of 38% under ACTD relative to ACTW, but no effect ECTD (Fig. 5B). In contrast, *CAT activity* did not vary irrespective of the treatment and species (Fig. 5 C and Table 1). Concerning *GR activity*, it was affected by the environmental treatment and the interaction among treatments in wheat, whereas in buckwheat it was affected by the water and the environmental treatments (Fig. 5D and Table 1). Upon exposure to ACTD, *GR activity* was significantly increased in wheat by 35% in comparison to ACTW, while under ECT it remained unchanged (Fig. 5D). In buckwheat, *GR activity* was 43% higher under ACTD compared to ACTW, and was significantly decreased by 42% under both ECT treatments (Fig. 5D).

Regarding antioxidant metabolites, *Total Glutathione* and *Total Ascorbate* were almost unchanged regardless of the treatment and the species (Fig. 5E-F and Table 1). In fact, we only found a significant increase of 33% in *Total Glutathione* in wheat under ECTW compared to ACTW (Fig. 5E), and an increase of *AsA* by 61% in buckwheat under ACTD in comparison to ACTW (Fig. 5 F).

3.5. Growth parameters

With respect to biomass accumulation, *DW* in wheat was affected by both treatments, while in buckwheat the water treatment was the main treatment that had an influence (Fig. 6A-D and Table 1). On the one hand, under ACTW, wheat exhibited a growth of 1.62 g and 2 g for *Leaf DW* and *Stem DW*, while these values were decreased by 73% and 67% upon exposure to ACTD (Fig. 6A-B). Buckwheat, on the other hand, showed *Leaf DW* and *Stem DW* growth of 2.31 g and 4.03 g under ACTW. While we found the growth for *Leaf DW* and *Stem DW* under ECTW and ECTD in wheat to be approximately 33% and 77% lower than under their corresponding ACT treatments, in buckwheat the growth of these organs was similar for the same comparison (Fig. 6A-B). Contrary to what occurred with aerial organs, *Root DW* was higher in wheat under ACT treatments than in buckwheat (Fig. 6 C). In spite of the aforementioned, when subjected to ECT treatments *Root DW* in wheat was decreased by 47% compared to ACTW, while in buckwheat it showed almost no variation (Fig. 6 C). Overall, wheat showed a *Total DW* of
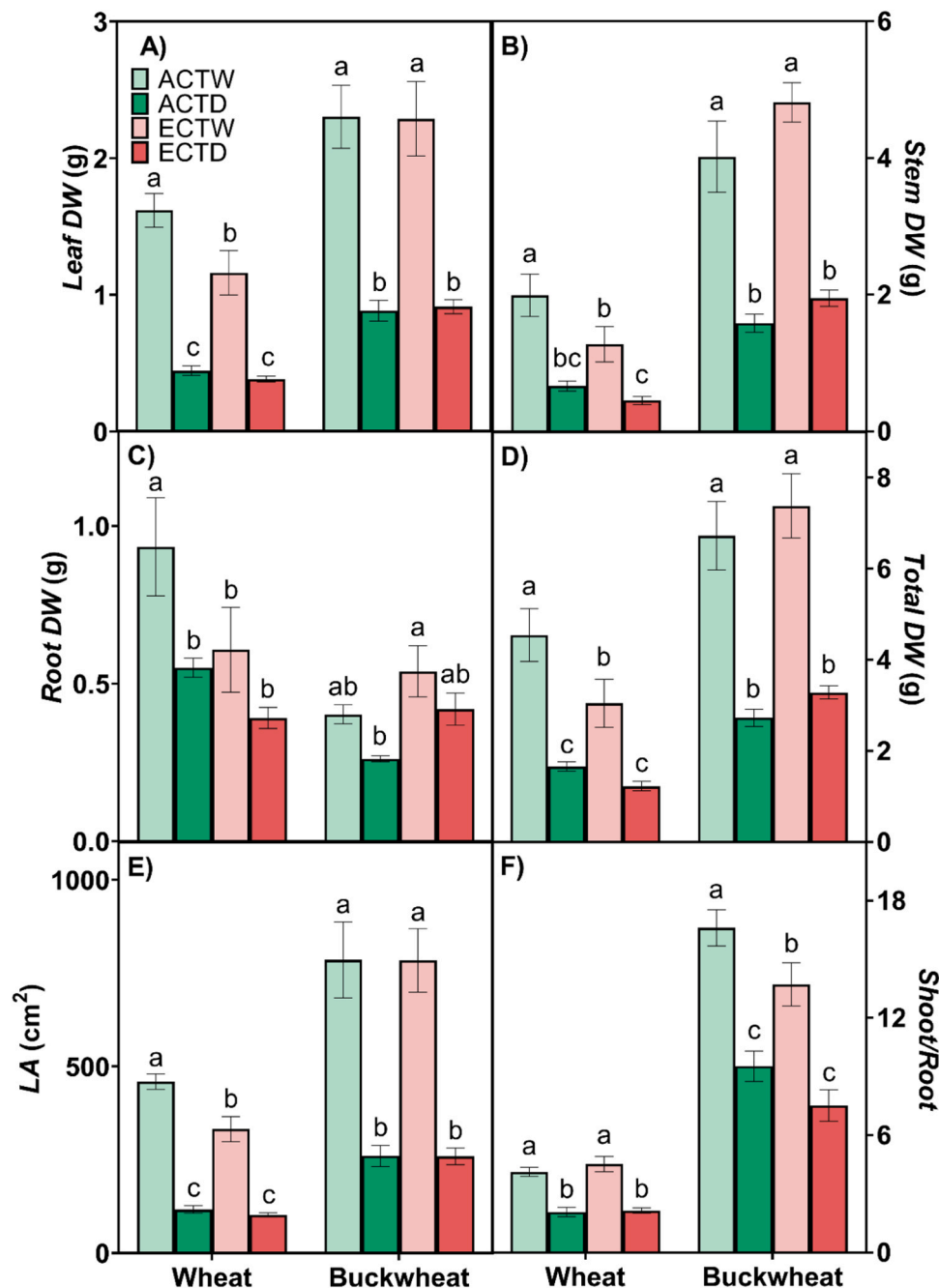
Fig. 6. Effect of ACTW, ACTD, ECTW and ECTD treatments in wheat and buckwheat in **A)** leaf dry-weight (*Leaf DW*) **B)** stem dry-weight (*Stem DW*), **C)** root dry-weight (*Root DW*), **D)** total dry-weight (*Total DW*), **E)** leaf area (*LA*) and **F)** *Shoot/Root*. Each bar represents mean ± S.E. of at least 6 replicates. Treatments and statistical analysis are explained in Fig. 1.

4.55 g, 1.66 g, 3.05 g and 1.23 g as opposed to the higher 6.73 g, 2.73 g, 7.38 g and 3.29 g in buckwheat under ACTW, ACTD, ECTW and ECTD, respectively (Fig. 6D). Unsurprisingly, *LA* showed a similar trend to the one observed in *Leaf DW* (Fig. 6E). As expected by the *Leaf DW*, *Stem DW* and *Root DW* results, both species had significantly lower *Shoot/Root* under drought compared to their respective well-watered treatments (Fig. 6 F and Table 1).

We found both treatments to influence *RGR* in wheat, while in buckwheat only water treatment had an effect (Fig. 7 A and Table 1). Overall, the highest *RGR* value for wheat was 131 mg DW g^{-1} DW day^{-1}, which was reached under ACTW (Fig. 7 A). In contrast, *RGR* values for buckwheat were on average 208 mg DW g^{-1} DW day^{-1} for W treatments and 168 mg DW g^{-1} DW day^{-1} for D treatments (Fig. 7 A).

When examining *NAR*, we found that wheat showed higher values under ACTW and ACTD in comparison to the corresponding ECT treatments, while on buckwheat values remained unchanged (Fig. 7B). Similarly, buckwheat had on average 24% and 100% higher values of *NAR* under ACT and ECT, respectively. *LAR* was affected by the water treatment in wheat, whereas in buckwheat both treatments had an influence (Fig. 7 C and Table 1). In fact, while in wheat the decreases in *LAR* were only observed under ACTD and ECTD, in buckwheat ECTW also decreased *LAR* (Fig. 7 C). Thus, when examining *NAR* and *LAR*, it becomes apparent that the *RGR* values were mainly driven by *LAR* in both species (Fig. 7A-C and Table 1). The only exception occurred under ECTW, where *RGR* appeared to be mainly driven by *NAR* in both, wheat and buckwheat. In like manner, the identical trend of *SLA* shows that *LAR*
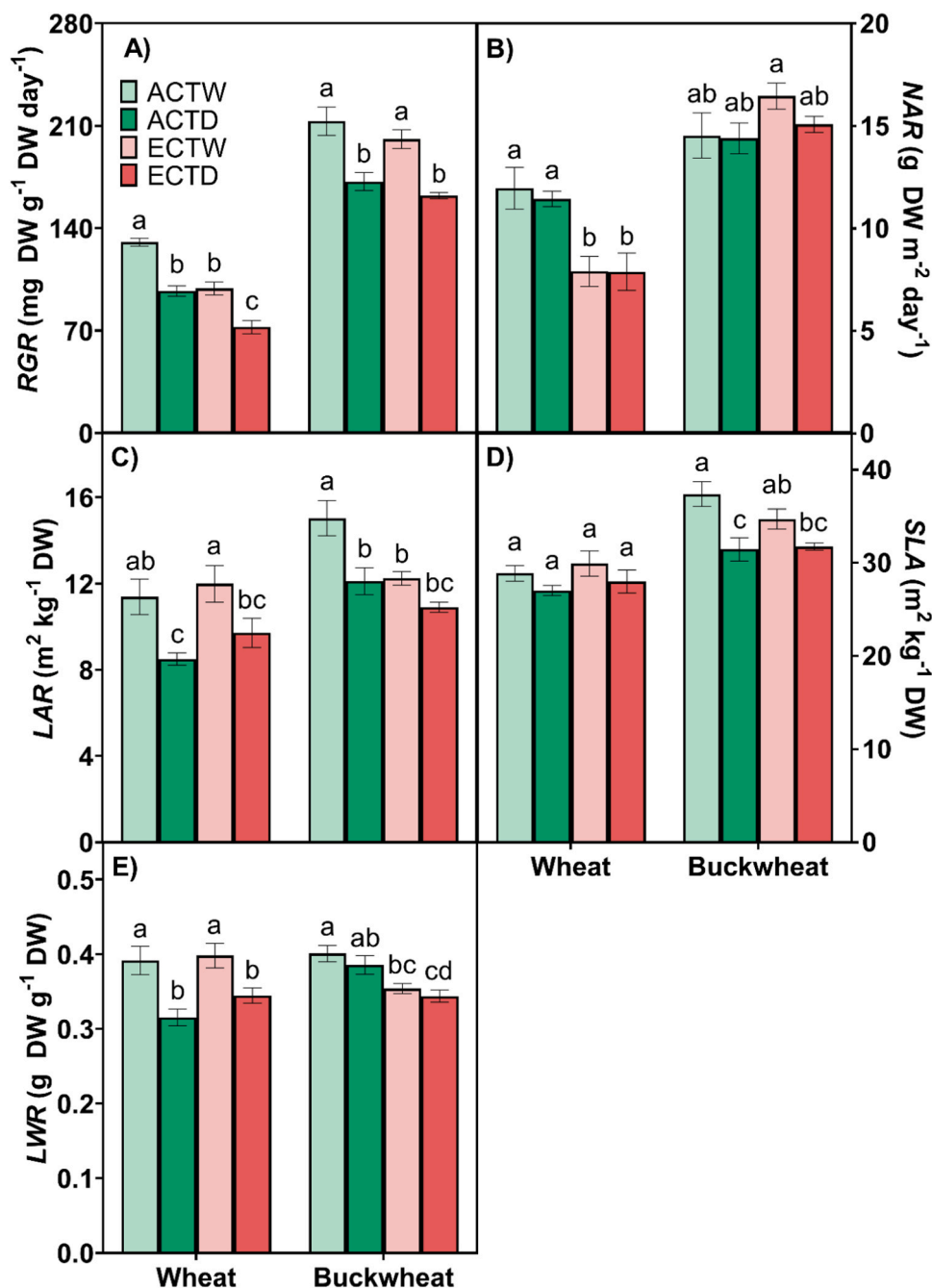
Fig. 7. Effect of ACTW, ACTD, ECTW and ECTD treatments in wheat and buckwheat in **A)** relative growth rate (*RGR*), **B)** net assimilation rate (*NAR*), **C)** leaf area ratio (*LAR*), **D)** specific leaf area (*SLA*), and **E)** leaf weight ratio (*LWR*). Each bar represents mean ± S.E. of at least 6 replicates. Treatments and statistical analysis are explained in Fig. 1.

was predominantly influenced by *SLA* in buckwheat, while it was mainly influenced by *LWR* in wheat (Fig. 7D-E and Table 1).

In both species *WUE* was influenced by the environmental treatment but not by the water treatment (Fig. 8 and Table 1). As a matter of fact, in wheat, *WUE* was only significantly increased from 3.60 g DW kg^{-1} H$_2$O under ACTW to 5.05 g DW kg^{-1} H$_2$O under ECTW (Fig. 8). Conversely, in buckwheat *WUE* showed significant increases under both, ECTW and ECTD, reaching values of approximately 6.40 g DW kg^{-1} H$_2$O compared to the 4.97 g DW kg^{-1} H$_2$O under ACTW (Fig. 8).

4. Discussion

The increasing environmental [CO$_2$] levels are expected to cause

higher global temperatures and more frequent drought episodes, generating an unfavourable environment for the growth of conventional crops (The Core Writing Team IPCC, 2021, 2015). In addition, food dependency to a small number of crops as wheat has put food security at risk as yields are expected to decline in the future (Ainsworth and Long, 2021; Li et al., 2021). In this context, buckwheat is presented as an alternative crop with the potential to cope with future climatic stresses and reduce food dependency while ensuring food supply (Martínez-Goñi et al., 2023a). Therefore, this research aims to characterise the growth of both wheat and buckwheat under four different environmental conditions, including: ambient [CO$_2$] and temperature (ACT) under well-watered (ACTW) or drought (ACTD) conditions, and high [CO$_2$] and temperature (ECT) under well-watered (ECTW) or drought (ECTD)
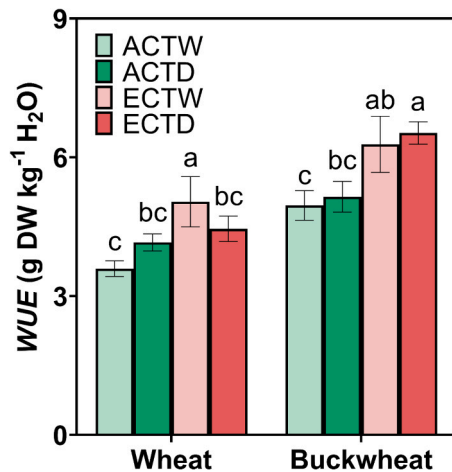
Fig. 8. Effect of ACTW, ACTD, ECTW and ECTD treatments in wheat and buckwheat in water-use efficiency (*WUE*). Each bar represents mean ± S.E. of at least 6 replicates. Treatments and statistical analysis are explained in Fig. 1.

conditions.

The ability to efficiently supply water within the plant when its availability is limited is crucial to ensure plant survival under drought. When drought is prolonged in time, plants decrease Ψ_w in order to ensure water supply inside their organs (Martínez-Vilalta and Garcia-Forner, 2017). This decrease may be driven by an active (osmotic adjustment) or passive (dehydration) increase of the osmolytes inside the cells, and the consequent decrease in Ψ_o (Fang and Xiong, 2015; Morgan, 1984). In our research, Ψ_w in wheat was passively decreased under ambient drought, as shown by the increases in *DH* (Table 2). Interestingly, we found high [CO_2] and high temperature to cause wheat to have similar water requirements as the ones observed under ambient drought. This was further confirmed by the equal increase in the *DH* level of wheat under ACTD and ECTW. Thus, a 3 °C rise in ambient temperature under high [CO_2] was sufficient to cause a dehydration level in wheat similar to that under ambient drought. This was likely attributed to the increase in leaf temperature resulting from the reduction in *gs* and *E* (Fig. 4A-B), which created an environment that promotes more rapid dehydration (Li et al., 2019). Upon exposure to future drought, the water requirements of wheat were extremely increased, as observed by the remarkable increase of *DH* and decrease of Ψ_w. Consequently, the greater passive decrease of Ψ_w was not sufficient to maintain water supply, as wheat relied on active mechanisms to ensure water uptake. In fact, wheat combined *OA* with increases in the cell wall elasticity, not only to promote water intake, but to maintain cell turgor (Miranda-Apodaca et al., 2018; Sanders and Arndt, 2012). All things considered, the activation of both passive and active mechanisms allowed wheat to preserve water inside the newer leaves as well as maintaining cell turgor, as shown by the steady *LRWC* and Ψ_p values across treatments. While these mechanisms were sufficient to maintain Ψ_o under future drought, there was a simultaneous synergistic increase in *DH*, resulting in other damages that will be discussed later. Regarding buckwheat, we found that it exhibited similar *LRWC* and *DH* values under well-watered conditions and the corresponding drought treatments, highlighting the ability of buckwheat to preserve water inside the leaves despite the water scarcity. Unlike wheat, buckwheat showed no signs of dehydration across the treatments. This suggests that drought, applied alone or in combination with high temperature and high [CO_2] failed to induce a detrimental impact on buckwheat.

Improved water management in crops is typically linked to the ability to restrict transpiration when exposed to water scarcity, thereby avoiding water losses (Burridge et al., 2022). Ambient drought caused *CuTr* to significantly decrease in both, wheat and buckwheat (Fig. 1). However, despite both species were capable of controlling their *CuTr*

when subjected to ambient drought, buckwheat managed to have a stricter regulation than wheat, as observed by the higher decreases in *CuTr*, explaining the better response of buckwheat compared to wheat. Buckwheat has been reported to be a drought-avoiding and water-saving species, with the ability to induce stomatal closure and decrease transpiration when drought is imposed (Aubert et al., 2021; Martínez-Goñi et al., 2023a). In our results, ambient drought caused an extreme reduction in the stomatal conductance and transpiration in wheat, while in buckwheat they remained unchanged. This was not unexpected, given that the lower *gs* and *E* values of buckwheat under ambient conditions enabled it to effectively preserve water without requiring to induce stomatal closure when subjected to water stress. In the same manner, high temperature and high [CO_2] have been reported to induce stomatal closure, leading to decreases in transpiration (Lahr et al., 2015; Shimono et al., 2010). Upon exposure to high temperature and high [CO_2], both species decreased their *gs* and *E*, even though in buckwheat the decrease was higher. This stricter regulation of stomata could explain the better water status in buckwheat and the increase in *DH* in wheat. Nevertheless, despite the major water-demanding environment generated by high [CO_2] and high temperature, buckwheat remained unaffected, reinforcing the previously discussed absence of stress on buckwheat in this environment. On the contrary, wheat reduced its *CuTr* even when water availability was not a problem, showing the susceptibility of wheat to the combination of high [CO_2] and high temperature.

Under future drought, *CuTr* was decreased to similar values to those under ambient drought condition in both species. In the same manner, future drought caused *gs* and *E* in wheat to reach similar values to those observed under ambient drought. On the contrary, in buckwheat, *gs* and *E* remained unchanged under future drought compared to future control conditions. Therefore, it appears that the constitutive reduced *gs* and *E* under high [CO_2] and high temperature allowed buckwheat to effectively conserve water even when future drought was imposed. Interestingly, even though buckwheat showed lower values of *gs* and *E* compared to wheat under both future conditions, our results may suggest that those lower values did not have an influence on *CuTr*. This apparent lack of correlation can be attributed to two potential factors. On the one hand, buckwheat had higher *LA* under high [CO_2] and high temperature when drought treatment was imposed (16.95 cm^2 in wheat and 27.50 cm^2 in buckwheat), which may have induced a greater transpiration in the early stages of the drought treatment. On the other hand, it is important to consider that the *gs* and *E* are measured at a specific point in time, whereas *CuTr* is based on a cumulative measurement over time. A plausible explanation is that the stomata of buckwheat plants gradually closed as the plants grew and the water stress progressed, resulting in a lower *gs* and *E* when they were measured. Conducting further research studying the early stomatal response to drought under high [CO_2] and high temperature conditions would provide valuable insights into this matter. Despite everything, the lower *gs* and *E* values observed in buckwheat allowed it to better regulate its *CuTr* when water scarcity was increased over time.

Moreover, the ability to better regulate water exchange capacity has been found to be correlated to stomatal distribution and morphology in plant leaves (Li et al., 2020; Richardson et al., 2017; Wall et al., 2022). When comparing the two species, buckwheat had higher *SD*, with especially higher proportion of SD_{AB} (Fig. 3). In contrast, wheat demonstrated a comparable proportion of stomata between the adaxial and abaxial surfaces. These results are not unexpected, since wheat is an amphistomatous species (R = 0.50), with a slightly higher proportion of SD_{AD} (Lu, 1989), while buckwheat has more hypostomatous characteristics (R = 0.28 in our results) (Martínez-Goñi et al., 2023a). Under ambient drought conditions, wheat showed an increase in SD_{AD} compared to ambient well-watered conditions, while buckwheat showed a remarkable increases in both, SD_{AD} and SD_{AB}. Likewise, SD_{AD} and SD_{AB} exhibited significant increases in buckwheat under future conditions, especially under future drought. Increasing *SD* in response to more water-demanding environments might appear as a

counterproductive mechanism in buckwheat, as an increase in stomata could potentially lead to higher transpiration rates and, consequently, greater water loss. Nonetheless, it is essential to consider stomatal size as another critical factor (Table 3). In fact, we found buckwheat to have smaller stomata regardless of the treatment. *SD* typically exhibits a negatively correlation with *S* during drought conditions, as *SD* tends to increase while *S* tends to decrease (Li et al., 2020). This is attributed to the fact that high densities of smaller stomata, as the ones observed in buckwheat, respond faster to water deficit, allowing an improved water-use efficiency and optimized long-term carbon gain (De Boer et al., 2012; Franks and Farquhar, 2007; Wall et al., 2023). Therefore, it becomes evident that buckwheat, in response to decreasing water availability, prioritizes the preservation of water resources over gas exchange capacity to ensure carbon gain in the long-term. Conversely, while wheat attempts a similar strategy, it is incapable of achieving a similar outcome.

All these variations in stomatal regulation directly affected the net photosynthetic [CO_2] assimilation rate, as well as the optimal status of the photosynthetic machinery (Tcherkez and Limami, 2019). As previously discussed, exposure to water scarcity induces stomatal closure as a short-term strategy to avoid water loss (Wall et al., 2006), but over prolonged periods could ultimately restrict photosynthesis (Silim et al., 2009). In our research, the decreases in *gs* and *E* in wheat under ambient drought caused *A* to significantly decrease (Fig. 4A-C), however Φ_{PSII} remained unaltered (Fig. 4D). As a consequence, *ETR/A* was increased (Fig. 4E), suggesting a disequilibrium in the NADP$^+$/NADPH recycling process (Halliwell and Gutteridge, 2015). As a result, the generation of the superoxide radical in the electron transport chain of the chloroplast increases, intensifying the risk of oxidative damage. Therefore, wheat upregulated *SOD activity* and *APX activity* as defence mechanisms to mitigate the overproduction of ROS (Fig. 5A-B) (Halliwell and Gutteridge, 2015). On the contrary, the absence of effect on *gs* and *E* in buckwheat allowed it to preserve its *A* regardless of the drought. Likewise, the sustained *ETR/A* suggests a more balanced NADP$^+$/NADPH recycling process and a lack of oxidative damage. However, we found *APX activity* to be increased in buckwheat. This upregulation could be attributed to the rise in *AsA* (Fig. 5 F), since promoting the production of antioxidant metabolites is more energy-efficient than increasing enzyme levels. Thus, buckwheat could be increasing *AsA* as a preventive mechanism for the forthcoming oxidative damage, leading to increases in *APX activity*. Future studies analysing the response of the antioxidant metabolism of buckwheat to drought could provide valuable insights into this matter.

Regarding high [CO_2] and high temperature, despite the reported decreases in *gs*, we found no significant effect on *A* in wheat compared to ambient conditions. Typically, high [CO_2] tends to increase *A* (Ainsworth and Long, 2021), so this apparent lack of effect is most likely attributed to the 3 °C temperature increase, which could be preventing high [CO_2] from effectively enhancing *A*. This would not be particularly surprising given that it has previously been reported 2 °C above ambient temperature to be the stress-threshold at which the mitigating effect of high [CO_2] is lost (Dias de Oliveira et al., 2013). Conversely, buckwheat exhibited improved *A* under the combination of high [CO_2] and high temperature, along with an enhanced Φ_{PSII} and decreased *ETR/A* (Fig. 4D-F). These results suggest that buckwheat will have the capacity to optimize its photosynthetic efficiency under the projected future conditions. Nevertheless, other dicotyledonous species, such as soybean, exhibited contrasting photosynthetic responses under high CO_2 and high temperature. In fact, while some researchers reported an increase in *A*, others found no effect or even a decrease (Ruiz-Vera et al., 2013; Thomey et al., 2019; Vu et al., 1997). This suggests that the positive effects reported on the photosynthesis of buckwheat under high CO_2 and high temperature might be variety-specific rather than species-specific. Therefore, further studies analysing the response of different buckwheat varieties could provide a more comprehensive understanding of this species response to the future climatic conditions.

When subjected to future drought, the decrease in *A* in wheat was even more substantial to the one observed under ambient drought. In addition, Φ_{PSII} in wheat was decreased, indicating signs of down-regulation or structural alteration (Foyer et al., 2017). Likewise, the consistent reduction in *A* and *ETR* could account for the similar reduction of *ETR/A* in wheat across different drought treatments. Consequently, even though the stress level in wheat under future drought was higher than under ambient drought, the oxidative damage and consequent upregulation of *SOD* and *APX* were similar to those observed under ambient drought. On the contrary, as occurred under future control conditions, buckwheat managed to increase *A* even when exposed to future drought. Contrary to what we found in wheat, the increase in *A* in buckwheat under the combination of the three environmental variables could be attributed to the fact that the 3 °C temperature increase is not sufficient to diminish the beneficial effect of high [CO_2] on *A*. Besides, buckwheat exhibited no down-regulation of Φ_{PSII} and lower *ETR/A* under future drought treatment. Thus, these results are consistent with the results discussed earlier for higher *SD* of smaller *S* in buckwheat.

Regarding growth, we found the growth of all organs in wheat to be significantly inhibited under ambient drought (Fig. 6A-D). Interestingly, although *A* was similar to that observed under ambient conditions, high temperature and high [CO_2] were capable of significantly inhibiting the growth of wheat. This could be attributed to increases in respiration promoted by high temperature, as it is expected that temperature effects will dominate when high [CO_2] and high temperature are combined (Dusenge et al., 2019; Yu et al., 2012). This response in total wheat growth under ECTW was consistent with the results discussed earlier, indicating that wheat exhibited a stress level that was not completely mitigated by high [CO_2] when temperature was increased by 3 °C. Upon exposure to future drought, total growth of wheat was decreased reaching similar values to those under ambient drought. These results align with previous research by Dias de Oliveira et al. (2013), who reported that the aboveground biomass of wheat under high [CO_2] levels in temperatures exceeding 2 °C was negatively affected, especially when it was also exposed to drought. Furthermore, alongside the decrease in aboveground biomass, we also found a reduction in *Root DW*. Thus, the ability to absorb water from the soil in wheat was decreased, indicating the requirement for a compensatory decrease in Ψ_w, as evidenced by the reported results. Therefore, the longer preservation of *SRWC* in wheat under ECTD could also be caused by its lack of ability to maintain adequate water uptake compared to ACTD. On the contrary, even though the growth in buckwheat was also inhibited by the ambient drought treatment, it remained significantly higher than in wheat. Moreover, buckwheat managed to preserve a similar growth to that observed under ambient conditions for both future conditions. Finding no variation in *Total DW* in buckwheat under ECT when compared to ACT was unexpected, since the higher *A* values for these treatments made us believe that the biomass accumulation would also be greater. However, as occurred in wheat, the extra fixed carbon in buckwheat under future conditions could have also been consumed via elevated respiration rates (Dusenge et al., 2019; Yu et al., 2012). Thus, even though buckwheat had a higher *A*, the increased levels of respiration may be inhibiting an increased *Total DW* outcome.

In the same manner, the discrepancy in *Total DW* among species could also be explained by different growth patterns when compared to the ambient conditions (Poorter, 1993). We found that the reported impact of ambient drought treatment in *RGR* was primarily explained by a decrease in *LAR* in wheat, with no variations in *NAR* (Fig. 7A-C). This reduction in *LAR* was primarily explained by a reduction in the allocation of resources to the leaves (*LWR*). These results are in agreement with those reported by Simane et al. (1993), who also found *RGR* to be mainly determined by *LAR* and its components in durum wheat (*Triticum turgidum*) when exposed to drought, with little or no influence of *NAR*. Under future control conditions, the decrease in *NAR* in wheat caused *RGR* to reach similar values to those under ambient drought, as *LAR*

remained unaffected. This was attributed to the decreased A of wheat under high $[CO_2]$ and high temperature, which suggests a limited ability of wheat to acclimate to this environment (Atkin et al., 2006). When subjected to future drought, RGR exhibited a more pronounced decrease in wheat, with LAR and NAR being both contributors to this decrease. This is not unexpected considering the fact that, in addition to the LA decreases, we also found strong gs and A reductions, as well as a Φ_{PSII} down-regulation. In contrast, as occurred in wheat, RGR was also decreased under ambient drought conditions in buckwheat. Nevertheless, even though in buckwheat this decrease was also explained by LAR, as opposed to wheat, the produced leaf area per leaf biomass (SLA) was identified to be the main limiting force and not LWR. Decreases in SLA, such as those observed in buckwheat, have been reported to provide greater plasticity to cope with environmental stresses, including drought (Krintza et al., 2024). Similarly, as opposed to ambient conditions, under future conditions RGR was determined by both, NAR and LAR. Overall, and irrespective of the treatment, buckwheat showed higher RGR compared to wheat, indicating that buckwheat exhibited superior growth, plasticity and biomass accumulation capabilities under similar conditions.

As a whole, our results show that the higher *Total DW* in buckwheat compared to wheat was mainly determined by variations in RGR. Moreover, the observed variations in RGR, together with the increases in respiration (Dusenge et al., 2019; Yu et al., 2012), would explain the reported decreases in *Total DW* in wheat under ECTW when A remained similar to that reported under ACTW. In addition, several parameters on gas exchange, antioxidant metabolism and whole growth in wheat suggested a worse performance compared to buckwheat, not just under ambient drought conditions, but also under future climatic conditions. Our results complement previous observations, where it was reported that wheat was negatively affected by increases in temperature above 2 °C despite high $[CO_2]$ (Dias de Oliveira et al., 2013). Likewise, we also found future drought to severely harm wheat, resulting in damages to its photosynthetic apparatus and significant limitations in growth. Buckwheat, on the contrary, showed an enhanced water status and use of water resources, which allowed it to withstand future drought. As a matter of fact, the higher WUE of buckwheat under ambient conditions was increased more than in wheat under the high $[CO_2]$ and high temperature (Fig. 8). High WUE is a particularly important trait in plants, as indicates an improved use of water resources that allow them to grow consuming less water and could ensure survival when crops are exposed to drought stress (Blankenagel et al., 2018; Condon et al., 2004; Rockström et al., 2007). It is important to note that although the present study has primarily examined the vegetative stage response of wheat and buckwheat, based projections for crop growth and yield under high temperatures and/or drought, as well as our own results, we believe that future climatic conditions might have a significant impact on the yield of wheat (Fahad et al., 2017; Zahra et al., 2021). In fact, Li et al. (2019) found that yield-related traits in wheat, such as spike number and thousand grain weight, were significantly decreased as a consequence of the combination of high temperature and drought, regardless of the atmospheric $[CO_2]$ levels. On the contrary, our research provides evidence on how the inherent capacity of buckwheat to use water resources more efficiently will be further enhanced under future climatic conditions. Therefore, we expect the impact on buckwheat yields to be less severe than in wheat. It is worth mentioning that, although some studies have analysed the individual effect of drought or high temperature on the reproductive stage of buckwheat (Aubert et al., 2021, 2020; Cawoy et al., 2006), analysing the combined impact of the three environmental variables of the future was beyond the scope of our current research. Thus, future studies should aim to examine the combined impact of high $[CO_2]$, high temperature and drought on the reproductive stage of buckwheat. Hence, our results confirm our main hypotheses, since wheat was severely damaged under the triple interaction compared to when exposed to the combination of high $[CO_2]$ and high temperature (confirmation of hypothesis 2). In the same manner, buckwheat was

capable of preserving its improved regulation of leaf transpiration and enhanced its photosynthetic activity under high $[CO_2]$ and high temperature, and exhibited resilience when exposed to future drought (confirmation of hypotheses 1 and 3).

CRediT authorship contribution statement

Xabier Simón Martínez de Goñi: Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Data curation, Conceptualization. **Jon Miranda-Apodaca:** Writing – review & editing, Methodology, Conceptualization. **Usue Pérez-López:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Xabier Simón Martínez de Goñi reports financial support was provided by Basque Government. Usue Pérez López reports financial support was provided by Basque Government. Jon Miranda Gonzalez de Apodaca reports financial support was provided by Basque Government. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

AbdElgawad, H., De Vos, D., Zinta, G., Domagalska, M.A., Beemster, G.T.S., Asard, H., 2015. Grassland species differentially regulate proline concentrations under future climate conditions: An integrated biochemical and modelling approach. N. Phytol. 208, 354–369. https://doi.org/10.1111/nph.13481.

Ainsworth, E.A., Long, S.P., 2021. 30 years of free-air carbon dioxide enrichment (FACE): What have we learned about future crop productivity and its potential for adaptation? Glob. Chang Biol. 27, 27–49. https://doi.org/10.1111/gcb.15375.

Atkin, O.K., Loveys, B.R., Atkinson, L.J., Pons, T.L., 2006. Phenotypic plasticity and growth temperature: Understanding interspecific variability. J. Exp. Bot. 57, 267–281. https://doi.org/10.1093/jxb/erj029.

Aubert, L., Konrádová, D., Kebbas, S., Barris, S., Quinet, M., 2020. Comparison of high temperature resistance in two buckwheat species *Fagopyrum esculentum* and *Fagopyrum tataricum*. J. Plant Physiol. 251, 153222 https://doi.org/10.1016/j.jplph.2020.153222.

Aubert, L., Konrádová, D., Barris, S., Quinet, M., 2021. Different drought resistance mechanisms between two buckwheat species *Fagopyrum esculentum* and *Fagopyrum tataricum*. Physiol. Plant 172, 577–586. https://doi.org/10.1111/ppl.13248.

Blankenagel, S., Yang, Z., Avramova, V., Schön, C.C., Grill, E., 2018. Generating plants with improved water use efficiency. Agronomy 8, 194. https://doi.org/10.3390/agronomy8090194.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem 72, 248–254. https://doi.org/10.1016/0003-2697(76)90527-3.

Burridge, J.D., Grondin, A., Vadez, V., 2022. Optimizing crop water use for drought and climate change adaptation requires a multi-scale approach. Front Plant Sci. 13, 1349. https://doi.org/10.3389/fpls.2022.824720.

Casado-García, A., Del-Canto, A., Sanz-Saez, A., Pérez-López, U., Bilbao-Kareaga, A., Fritschi, F.B., Miranda-Apodaca, J., Muñoz-Rueda, A., Sillero-Martínez, A., Yoldi-Achalandabaso, A., Lacuesta, M., Heras, A., 2020. LabelStoma: A tool for stomata detection based on the YOLO algorithm. Comput. Electron Agric. 178, 105751 https://doi.org/10.1016/j.compag.2020.105751.

Cawoy, V., Lutts, S., Kinet, J.M., 2006. Osmotic stress at seedling stage impairs reproductive development in buckwheat (*Fagopyrum esculentum*). Physiol. Plant 128, 689–700. https://doi.org/10.1111/j.1399-3054.2006.00801.x.

Cheng, A., 2018. Review: Shaping a sustainable food future by rediscovering long-forgotten ancient grains. Plant Sci. 269, 136–142. https://doi.org/10.1016/j.plantsci.2018.01.018.

Condon, A.G., Richards, R.A., Rebetzke, G.J., Farquhar, G.D., 2004. Breeding for high water-use efficiency. J. Exp. Bot. 55, 2447–2460. https://doi.org/10.1093/jxb/erh277.

De Boer, H.J., Eppinga, M.B., Wassen, M.J., Dekker, S.C., 2012. A critical transition in leaf evolution facilitated the Cretaceous angiosperm revolution. Nat. Commun. 3, 1221. https://doi.org/10.1038/ncomms2217.

Delpérée, C., Kinet, J.M., Lutts, S., 2003. Low irradiance modifies the effect of water stress on survival and growth-related parameters during the early developmental stages of buckwheat (*Fagopyrum esculentum*). Physiol. Plant 119, 211–220. https://doi.org/10.1034/j.1399-3054.2003.00170.x.

Dias de Oliveira, E., Bramley, H., Siddique, K.H.M., Henty, S., Berger, J., Palta, J.A., 2013. Can elevated CO_2 combined with high temperature ameliorate the effect of terminal drought in wheat? Funct. Plant Biol. 40, 160–171. https://doi.org/10.1071/FP12206.

Dias de Oliveira, E., Palta, J.A., Bramley, H., Stefanova, K., Siddique, K.H.M., 2015. Elevated CO_2 reduced floret death in wheat under warmer average temperatures and terminal drought. Front Plant Sci. 6, 1–12. https://doi.org/10.3389/fpls.2015.01010.

Dikšaitytė, A., Viršilė, A., Žaltauskaitė, J., Januškaitienė, I., Juozapaitienė, G., 2019. Growth and photosynthetic responses in *Brassica napus* differ during stress and recovery periods when exposed to combined heat, drought and elevated CO2. Plant Physiol. Biochem. 142, 59–72. https://doi.org/10.1016/j.plaphy.2019.06.026.

Drake, B.G., Gonzàlez-Meler, M.A., Long, S.P., 1997. More efficient plants: A consequence of rising atmospheric CO_2? Annu. Rev. Plant Biol. 48, 609–639. https://doi.org/10.1146/annurev.arplant.48.1.609.

Dusenge, M.E., Duarte, A.G., Way, D.A., 2019. Plant carbon metabolism and climate change: elevated CO_2 and temperature impacts on photosynthesis, photorespiration and respiration. N. Phytol. 221, 32–49. https://doi.org/10.1111/nph.15283.

Edwards, E.A., Rawsthorne, S., Mullineaux, P.M., 1990. Subcellular distribution of multiple forms of glutathione reductase in leaves of pea (*Pisum sativum* L.). Planta 180, 278–284. https://doi.org/10.1007/BF00194008.

Fahad, S., Bajwa, A.A., Nazir, U., Anjum, S.A., Farooq, A., Zohaib, A., Sadia, S., Nasim, W., Adkins, S., Saud, S., Ihsan, M.Z., Alharby, H., Wu, C., Wang, D., Huang, J., 2017. Crop production under drought and heat stress: Plant responses and management options. Front Plant Sci. 8, 1147. https://doi.org/10.3389/fpls.2017.01147.

Fang, Y., Xiong, L., 2015. General mechanisms of drought response and their application in drought resistance improvement in plants. Cell. Mol. Life Sci. 72, 673–689. https://doi.org/10.1007/s00018-014-1767-0.

FAOSTAT, 2021. FAOSTAT statistical database [WWW Document]. URL https://www.fao.org/faostat/en/#home (accessed 3.6.23).

Farooq, M., Wahid, A., Kobayashi, N., Fujita, D., Basra, S.M.A., 2009. Plant drought stress: Effects, mechanisms and management. Agron. Sustain Dev. 29, 185–212. https://doi.org/10.1051/agro:2008021.

Foyer, C.H., Ruban, A.V., Noctor, G., 2017. Viewing oxidative stress through the lens of oxidative signalling rather than damage. Biochem. J. 474, 877–883. https://doi.org/10.1042/BCJ20160814.

Franks, P.J., Farquhar, G.D., 2007. The mechanical diversity of stomata and its significance in gas-exchange control. Plant Physiol. 143, 78–87. https://doi.org/10.1104/pp.106.089367.

Garg, M., Sharma, A., Vats, S., Tiwari, V., Kumari, A., Mishra, V., Krishania, M., 2021. Vitamins in cereals: A critical review of content, health effects, processing losses, bioaccessibility, fortification, and biofortification strategies for their improvement. Front Nutr. 8, 254. https://doi.org/10.3389/fnut.2021.586815.

Griffith, O.W., 1980. Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinylpyridine. Anal. Biochem 106, 207–212. https://doi.org/10.1016/0003-2697(80)90139-6.

Halliwell, B., Gutteridge, J.M.C., 2015. Free radicals in biology and medicine. Free Radicals in Biology and Medicine, 5th ed. Oxford Academic. https://doi.org/10.1093/acprof:oso/9780198717478.001.0001.

Hoagland, D.R., Arnon, D.I., 1938. The water-culture method for growing plants without soil. Calif. Agric. Exp. Station Circ. 32.

Hossain, M.A., Asada, K., 1984. Inactivation of ascorbate peroxidase in spinach chloroplasts on dark addition of hydrogen peroxide: Its protection by ascorbate. Plant Cell Physiol. 25, 1285–1295. https://doi.org/10.1093/oxfordjournals.pcp.a076837.

Huang, B., Liu, X., Xu, Q., 2001. Supraoptimal soil temperatures induced oxidative stress in leaves of creeping bentgrass cultivars differing in heat tolerance. Crop Sci. 41, 430–435. https://doi.org/10.2135/cropsci2001.412430x.

Kacienė, G., Miškelytė, D., AbdElgawad, H., Beemster, G., Asard, H., Dikšaitytė, A., Žaltauskaitė, J., Sujetovienė, G., Januškaitienė, I., Juknys, R., 2019. O₃ pollution in a future climate increases the competition between summer rape and wild mustard. Plant Physiol. Biochem. 135, 194–205. https://doi.org/10.1016/j.plaphy.2018.11.031.

Kimball, B.A., 2016. Crop responses to elevated CO_2 and interactions with H_2O, N, and temperature. Curr. Opin. Plant Biol. 31, 36–43. https://doi.org/10.1016/j.pbi.2016.03.006.

Krintza, N., Dener, E., Seifan, M., 2024. Stress induces trait variability across multiple spatial scales in the arid annual plant *Anastatica hierochuntica*. Plants 13, 256. https://doi.org/10.3390/plants13020256.

Ku, S.-B., Edwards, G.E., 1977b. Oxygen Inhibition of Photosynthesis: II. Kinetic Characteristics as Affected by Temperature 1. Plant Physiol. 59, 991–999. https://doi.org/10.1104/PP.59.5.991.

Ku, S.-B., Edwards, G.E., 1977a. Oxygen Inhibition of Photosynthesis. Plant Physiol. 59, 986–990. https://doi.org/10.1104/pp.59.5.991.

Lahr, E.C., Schade, G.W., Crossett, C.C., Watson, M.R., 2015. Photosynthesis and isoprene emission from trees along an urban-rural gradient in Texas. Glob. Chang Biol. 21, 4221–4236. https://doi.org/10.1111/gcb.13010.

Lee, J.S., 2011. Combined effect of elevated CO_2 and temperature on the growth and phenology of two annual C_3 and C_4 weedy species. Agric. Ecosyst. Environ. 140, 484–491. https://doi.org/10.1016/j.agee.2011.01.013.

Li, P., Ma, B., Palta, J.A., Ding, T., Cheng, Z., Lv, G., Xiong, Y., 2021. Wheat breeding highlights drought tolerance while ignores the advantages of drought avoidance: A meta-analysis. Eur. J. Agron. 122, 126196 https://doi.org/10.1016/j.eja.2020.126196.

Li, S., Li, X., Wei, Z., Liu, F., 2020. ABA-mediated modulation of elevated CO_2 on stomatal response to drought. Curr. Opin. Plant Biol. 56, 174–180. https://doi.org/10.1016/j.pbi.2019.12.002.

Li, X., Kristiansen, K., Rosenqvist, E., Liu, F., 2019. Elevated CO_2 modulates the effects of drought and heat stress on plant water relations and grain yield in wheat. J. Agron. Crop Sci. 205, 362–371. https://doi.org/10.1111/jac.12330.

Lu, Z.M., 1989. Ratio of stomatal resistance on two sides of wheat leaves as affected by soil water content. Agric. Meteor. 49, 1–7. https://doi.org/10.1016/0168-1923(89)90057-9.

Mahrookashani, A., Siebert, S., Hüging, H., Ewert, F., 2017. Independent and combined effects of high temperature and drought stress around anthesis on wheat. J. Agron. Crop Sci. 203, 453–463. https://doi.org/10.1111/jac.12218.

Martínez-Goñi, X.S., Robredo, A., Pérez-López, U., Muñoz-Rueda, A., Mena-Petite, A., 2023b. *Sorghum bicolor* prioritizes the recovery of its photosynthetic activity when re-watered after severe drought stress, while manages to preserve it under elevated CO_2 and drought. J. Agron. Crop Sci. 209, 217–227. https://doi.org/10.1111/jac.12618.

Martínez-Goñi, X.S., Miranda-Apodaca, J., Pérez-López, U., 2023a. Could buckwheat and spelt be alternatives to wheat under future environmental conditions? Study of their physiological response to drought. Agric. Water Manag 278, 108176. https://doi.org/10.1016/J.AGWAT.2023.108176.

Martínez-Vilalta, J., Garcia-Forner, N., 2017. Water potential regulation, stomatal behaviour and hydraulic transport under drought: deconstructing the iso/anisohydric concept. Plant Cell Environ. 40, 962–976. https://doi.org/10.1111/pce.12846.

Mathur, S., Agrawal, D., Jajoo, A., 2014. Photosynthesis: Response to high temperature stress. J. Photochem. Photobio. B 137, 116–126. https://doi.org/10.1016/j.jphotobiol.2014.01.010.

McCord, J.M., Fridovich, I., 1969. Superoxide dismutase. An enzymic function for erythrocuprein (hemocuprein). J. Biol. Chem. 244, 6049–6055. https://doi.org/10.1016/S0021-9258(18)63504-5.

Miranda-Apodaca, J., Pérez-López, U., Lacuesta, M., Mena-Petite, A., Muñoz-Rueda, A., 2018. The interaction between drought and elevated CO_2 in water relations in two grassland species is species-specific. J. Plant Physiol. 220, 193–202. https://doi.org/10.1016/j.jplph.2017.11.006.

Morgan, J.M., 1984. Osmoregulation and water stress in higher plants. Annu Rev. Plant Physiol. 35, 299–319. https://doi.org/10.1146/annurev.pp.35.060184.001503.

Nguyen, T., Bajwa, A.A., Navie, S., O'Donnell, C., Adkins, S., 2017. Parthenium weed (*Parthenium hysterophorus* L.) and climate change: the effect of CO_2 concentration, temperature, and water deficit on growth and reproduction of two biotypes. Environ. Sci. Pollut. Res. 24, 10727–10739. https://doi.org/10.1007/s11356-017-8737-7.

O'Leary, G.J., Christy, B., Nuttall, J., Huth, N., Cammarano, D., Stöckle, C., Basso, B., Shcherbak, I., Fitzgerald, G., Luo, Q., Farre-Codina, I., Palta, J., Asseng, S., 2015. Response of wheat growth, grain yield and water use to elevated CO_2 under a Free-Air CO_2 Enrichment (FACE) experiment and modelling in a semi-arid environment. Glob. Chang Biol. 21, 2670–2686. https://doi.org/10.1111/gcb.12830.

Pérez-López, U., Robredo, A., Lacuesta, M., Sgherri, C., Muñoz-Rueda, A., Navari-Izzo, F., Mena-Petite, A., 2009. The oxidative stress caused by salinity in two barley cultivars is mitigated by elevated CO_2. Physiol. Plant 135, 29–42. https://doi.org/10.1111/j.1399-3054.2008.01174.x.

Pérez-López, U., Robredo, A., Lacuesta, M., Sgherri, C., Mena-Petite, A., Navari-Izzo, F., Muñoz-Rueda, A., 2010. Lipoic acid and redox status in barley plants subjected to salinity and elevated CO_2. Physiol. Plant 139, 256–268. https://doi.org/10.1111/j.1399-3054.2010.01361.x.

Poorter, H., 1993. Interspecific variation in the growth response of plants to an elevated ambient CO_2 concentration. Vegetatio 104–105 77–97. https://doi.org/10.1007/BF00048146.

Prasad, P.V.V., Pisipati, S.R., Ristic, Z., Bukovnik, U., Fritz, A.K., 2008. Impact of night time temperature on physiology and growth of spring wheat. Crop Sci. 48, 2372–2380. https://doi.org/10.2135/cropsci2007.12.0717.

Prasad, P.V.V., Pisipati, S.R., Momčilović, I., Ristic, Z., 2011. Independent and combined effects of high temperature and drought stress during grain filling on plant yield and chloroplast EF-Tu expression in spring wheat. J. Agron. Crop Sci. 197, 430–441. https://doi.org/10.1111/j.1439-037X.2011.00477.x.

Reddy, A.R., Chaitanya, K.V., Vivekanandan, M., 2004. Drought-induced responses of photosynthesis and antioxidant metabolism in higher plants. J. Plant Physiol. 161, 1189–1202. https://doi.org/10.1016/j.jplph.2004.01.013.

Richardson, F., Brodribb, T.J., Jordan, G.J., 2017. Amphistomatic leaf surfaces independently regulate gas exchange in response to variations in evaporative demand. Tree Physiol. 37, 869–878. https://doi.org/10.1093/treephys/tpx073.

Rockström, J., Lannerstad, M., Falkenmark, M., 2007. Assessing the water challenge of a new green revolution in developing countries. Proc. Natl. Acad. Sci. USA 104, 6253–6260. https://doi.org/10.1073/pnas.0605739104.

Rodríguez, J.P., Rahman, H., Thushar, S., Singh, R.K., 2020. Healthy and resilient cereals and pseudo-cereals for marginal agriculture: molecular advances for improving nutrient bioavailability. Front Genet 11, 49. https://doi.org/10.3389/fgene.2020.00049.

Ruiz-Vera, U.M., Siebers, M., Gray, S.B., Drag, D.W., Rosenthal, D.M., Kimball, B.A., Ort, D.R., Bernacchi, C.J., 2013. Global warming can negate the expected CO_2

stimulation in photosynthesis and productivity for soybean grown in the midwestern United States. Plant Physiol. 162, 410–423. https://doi.org/10.1104/pp.112.211938.

Sanders, G.J., Arndt, S.K., 2012. Osmotic adjustment under drought conditions. In: Plant Responses to Drought Stress: From Morphological to Molecular Features. Springer-Verlag, Berlin Heidelberg, pp. 199–229. https://doi.org/10.1007/978-3-642-32653-0_8.

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., Tinevez, J.Y., White, D.J., Hartenstein, V., Eliceiri, K., Tomancak, P., Cardona, A., 2012. Fiji: An open-source platform for biological-image analysis. Nat. Methods 9, 676–682. https://doi.org/10.1038/nmeth.2019.

Schindler, C., Lichtenthaler, H.K., 1996. Photosynthetic CO_2-assimilation, chlorophyll fluorescence and zeaxanthin accumulation in field grown maple trees in the course of a sunny and a cloudy day. J. Plant Physiol. 148, 399–412. https://doi.org/10.1016/S0176-1617(96)80272-0.

Scholander, P.F., Hammel, H.T., Bradstreet, E.D., Hemmingsen, E.A., 1965. Sap pressure in vascular plants. Science 148 (1979), 339–346. https://doi.org/10.1126/science.148.3668.339.

Shimono, H., Okada, M., Inoue, M., Nakamura, H., Kobayashi, K., Hasegawa, T., 2010. Diurnal and seasonal variations in stomatal conductance of rice at elevated atmospheric CO_2 under fully open-air conditions. Plant Cell Environ. 33, 322–331. https://doi.org/10.1111/j.1365-3040.2009.02057.x.

Silim, S., Nash, R., Reynard, D., White, B., Schroeder, W., 2009. Leaf gas exchange and water potential responses to drought in nine poplar (*Populus* spp.) clones with contrasting drought tolerance. Trees - Struct. Funct. 23, 959–969. https://doi.org/10.1007/S00468-009-0338-8/FIGURES/5.

Simane, B., Peacock, J.M., Struik, P.C., 1993. Differences in developmental plasticity and growth rate among drought-resistant and susceptible cultivars of durum wheat (*Triticum turgidum* L. var. *durum*). Plant Soil 157, 155–166. https://doi.org/10.1007/BF00011044.

Tcherkez, G., Limami, A.M., 2019. Net photosynthetic CO_2 assimilation: more than just CO_2 and O_2 reduction cycles. N. Phytol. 223, 520–529. https://doi.org/10.1111/nph.15828.

The Core Writing Team IPCC, 2015. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change, Climate Change 2014: Synthesis Report. IPCC.

The Core Writing Team IPCC, 2021. Working Group I Contribution to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change, Climate Change 2021: The Physical Science Basis. IPCC.

Thomey, M.L., Slattery, R.A., Köhler, I.H., Bernacchi, C.J., Ort, D.R., 2019. Yield response of field-grown soybean exposed to heat waves under current and elevated [CO_2. Glob. Chang Biol. 25, 4352–4368. https://doi.org/10.1111/gcb.14796.

von Caemmerer, S., Farquhar, G.D., 1981. Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. Planta 153, 376–387. https://doi.org/10.1007/BF00384257.

Vu, J.C.V., Allen, L.H., Boote, K.J., Bowes, G., 1997. Effects of elevated CO_2 and temperature on photosynthesis and Rubisco in rice and soybean. Plant Cell Environ. 20, 68–76. https://doi.org/10.1046/j.1365-3040.1997.d01-10.x.

Wall, G.W., Garcia, R.L., Kimball, B.A., Hunsaker, D.J., Pinter, P.J., Long, S.P., Osborne, C.P., Hendrix, D.L., Wechsung, F., Wechsung, G., Leavitt, S.W., LaMorte, R. L., Idso, S.B., 2006. Interactive effects of elevated carbon dioxide and drought on wheat. Agron. J. 98, 354–381. https://doi.org/10.2134/agronj2004.0089.

Wall, S., Vialet-Chabrand, S., Davey, P., Van Rie, J., Galle, A., Cockram, J., Lawson, T., 2022. Stomata on the abaxial and adaxial leaf surfaces contribute differently to leaf gas exchange and photosynthesis in wheat. N. Phytol. 235, 1743–1756. https://doi.org/10.1111/nph.18257.

Wall, S., Cockram, J., Vialet-Chabrand, S., Van Rie, J., Gallé, A., Lawson, T., 2023. The impact of growth at elevated [CO_2] on stomatal anatomy and behavior differs between wheat species and cultivars. J. Exp. Bot. 74, 2860–2874. https://doi.org/10.1093/jxb/erad011.

Yu, J., Chen, L., Xu, M., Huang, B., 2012. Effects of elevated CO_2 on physiological responses of tall fescue to elevated temperature, drought stress, and the combined stresses. Crop Sci. 52, 1848–1858. https://doi.org/10.2135/cropsci2012.01.0030.

Zahra, N., Wahid, A., Hafeez, M.B., Ullah, A., Siddique, K.H.M., Farooq, M., 2021. Grain development in wheat under combined heat and drought stress: Plant responses and management. Environ. Exp. Bot. 188, 104517 https://doi.org/10.1016/j.envexpbot.2021.104517.

Zinta, G., AbdElgawad, H., Domagalska, M.A., Vergauwen, L., Knapen, D., Nijs, I., Janssens, I.A., Beemster, G.T.S., Asard, H., 2014. Physiological, biochemical, and genome-wide transcriptional analysis reveals that elevated CO_2 mitigates the impact of combined heat wave and drought stress in Arabidopsis thaliana at multiple organizational levels. Glob. Chang Biol. 20, 3670–3685. https://doi.org/10.1111/gcb.12626.