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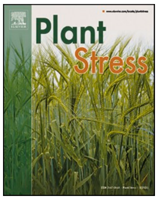

C and N allocation on wheat under the effects of depleted, current and elevated $[CO_2]$ are modulated by water availability

Salvador Aljazairi [a,b,c,*], Brigen Manikan [a], Xavier Serrat [a], Salvador Nogués [a]

[a] *Departament de Biologia Evolutiva, Ecologia i Ciències ambientals, Secció de Fisiologia Vegetal, Universitat de Barcelona, 08028 Barcelona, Catalonia, Spain*
[b] *Departamento de Ecología, Facultad de Ciencias, Universidad de Granada, 18071 Granada, Spain*
[c] *Departamento de Educación de Ciencias Experimentales, Universidad Internacional de la Rioja, UNIR, Spain*

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ABSTRACT

Although elevated atmospheric $[CO_2]$ has substantial indirect effects on vegetation carbon uptake via associated climate change, their dynamics remain unclear. The carbon and nitrogen allocation and partitioning in durum wheat were compared at different $[CO_2]$ and different water availability. The aim of this study was to investigate how the impacts of depleted and elevated $[CO_2]$ driven climate change on Mediterranean wheat plants under drought conditions. For that reason, double stable isotope labelling using $^{13}CO_2$ and $^{15}NH_4$–$^{15}NO_3$ was conducted to follow ^{13}C and ^{15}N allocation and partitioning in the different plant organs. Plants were studied in growth chambers under three different CO_2 environments (depleted, current and elevated) and two water availability conditions (well-watered and mild-water-stress). Isotopic ^{13}C and ^{15}N determination, gas exchange analyses and growth parameters were measured.

We show that plants subjected to depleted and elevated $[CO_2]$ suffered up and down regulation of photosynthesis respectively, but their responses were both modulated by water availability. Depleted $[CO_2]$ and drought reduced plant biomass. However, elevated $[CO_2]$, show that the initial positive effect of elevated $[CO_2]$ on carbon uptake declined rapidly, showing a consequence of physiological acclimation and the inhibition of [Rubisco] and activity, this effect was more evident in combination with drought. In both cases, depleted $[CO_2]$ and elevated $[CO_2]$ condition modified the C and N allocation compared with current $[CO_2]$, overall combined with drought.

These results obtained highlight the different C and N management strategies of wheat and provide relevant information about the potential response of plants under global climate change conditions.

Introduction

Climate models have projected that the climate change will have a significant impact on climate, plant function, and agriculture, strongly reducing crop yields by 2050 (IPCC, 2007; Wing et al., 2021). The rapid increase in the concentration of atmospheric CO_2 due to continued anthropogenic emissions of greenhouse gases in combination with loss of biodiversity and change land use are the main factors driving global climate change (Jaureguiberry et al., 2022). Terrestrial ecosystems absorb −30 % of anthropogenic CO_2 emissions and thus play a fundamental role in climate change (Chen et al., 2024) Atmospheric $[CO_2]$ remained stable at 260 ppm for thousands of years before the Industrial Revolution. However, since then, CO_2 has steadily accumulated in the atmosphere. The global atmospheric $[CO_2]$ has increased from its pre-industrial and continues to increase at a rate of about 2 ppm per year (Pagani et al., 1999; Pearson and Palmer, 2000; Tans, 2009). The CO_2 level reached 418.82 ppm in December 2022 (NOAA, 2023). By the end of this century, CO_2 concentration is projected to reach 700 ppm (Oldeman et al., 2024; Prentice et al., 2001).

Moreover, model predictions also indicate that by the end of the century, temperatures will increase by between four and five degrees, along with changes in the amount and frequency of rainfall and the

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Abbreviations: $\delta^{13}C$, carbon isotope composition; $\Delta^{13}C$, carbon isotope discrimination; A_n, net photosynthetic rate; C_{new}, new labeled C in respired CO_2; IRMS, isotope ratio mass spectrometry; PPFD, photosynthetic photon flux density; R_d, dark respiration; TOM, total organic matter; WS, mild water stress; WW, well water.

* Corresponding author at: Departament de Biologia Evolutiva, Ecologia i Ciències ambientals, Secció de Fisiologia Vegetal, Universitat de Barcelona, 08028 Barcelona, Catalonia, Spain.

E-mail address: sal@ugr.es (S. Aljazairi).

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intensity of tropical cyclones, droughts, and heat waves (IPCC, 2013). However, new literature about climatic future scenarios is appearing with a contribution of climatic mitigation to reduce the increasing of temperature (Kikstra et al., 2022). Drought is a climatic phenomenon that can occur periodically in all climatic zones, but occurs with greater frequency in tropical, sub-tropical and Mediterranean climatic regions, causing physiological damage to plants in ecosystems and agro-ecosystems (Ciriaco da Silva et al., 2011). Drought affects both plant hydraulics and carbon balance because plants respond to decreasing soil water availability by reducing stomatal conductance, thereby reducing carbon assimilation rates (Yang et al., 2024; Brodribb and McAdam, 2011). In addition, prolonged droughts cause declines in plant water potential (Mitchell et al., 2013), which negatively affect plant phloem functioning (Holtta et al., 2009) and can inhibit the mobilization and translocation of stored carbon from source to sink tissues (Lal et al., 2022; Sala et al., 2010; Hartmann et al., 2013).

The rate of CO_2 assimilation by plants is directly influenced by atmospheric [CO_2] (Lorenz and Lal, 2010). Besides, CO_2 availability also modulates the effects of drought on plants. It has been reported that elevated CO_2 alleviates the effects of drought stress, because transpiration is reduced when the stomata are partly closed (Cao et al., 2022; AbdElgawad et al., 2023), however, some of the mechanisms underlying these effects remain unclear (AbdElgawad et al., 2015; Aljazairi and Nogués, 2015; Wang et al., 2022). Therefore, it is essential to study how plants adapt from depleted [CO_2] to current [CO_2]. These adaptations may help to understand how plants would respond to future increases in [CO_2] combined with water deficits (Sage and Coleman, 2001; Nogués and Azcón-Bieto, 2013; Aljazairi et al., 2014b; Aljazairi and Nogués, 2015). Changes in precipitation associated with continued CO_2 emissions will bring changes in land suitability and wheat yields (IPCC, 2013). The effect of drought on yield is mediated, to a considerable extent, by changes in photosynthetic activity and stomatal conductance. Thus, the increases of atmospheric [CO_2], temperature, and changes in precipitation, have the potential to affect C_3, C_4, or CAM photosynthesis, however, that changes in the atmospheric CO_2 levels will more likely influence the photosynthetic activities of C_3 than of C_4 and CAM plants (Ehleringer, 2005), specially in wheat (IFPRI, 2013), since the productivity and quality of this crop is greatly affected by environmental conditions, with water stress being the main abiotic constraint on productivity (Araus et al., 2002; Dettori et al., 2022). Wheat is one of the most important food crops to human populations as it is consumed worldwide (Igrejas and Branlard, 2020).

Plants control their stomata to regulate water transpiration, which is modulated by [CO_2] and water availability in the environment. Furthermore, the ability to accurately measure the CO_2 responses of stomatal conductance, canopy evapotranspiration, and soil moisture is an important component of climate change studies (Leakey et al., 2009; Wang et al., 2022). Atmospheric CO_2 reductions can enhance stress intensity because at depleted [CO_2], plants tend to open stomata and exacerbate the limitations associated with drought (Sage and Cowling, 1999; Sage and Coleman, 2001; Marcinkowski and Piniewski, 2024). In contrast, under elevated [CO_2], plants have lower stomatal conductance which should ameliorate water deficits and, in some cases, significantly increase plant growth under drought conditions (Morgan et al., 2004, 2011; Perry et al., 2013; Alza et al., 2024). The opposite effect was found in other studies that have reported that elevated [CO_2] can compensate for water deficit inducing reduction in growth (Aranjuelo et al., 2009a). Thus, elevated [CO_2] also increases water use efficiency (WUE) in many plants under drought conditions, reducing their demand for water (Ainsworth and Long, 2005; Erice et al., 2007). That being said, knowledge of the mechanisms the interaction between depleted, current, and enriched CO_2 and water deficit should be receive more attention, as well as studies on photosynthesis and chlorophyll fluorescence, specially, in cereals (Lopes et al., 2004; Islam and Khan, 2019) and is a matter of a great concern for the understanding of plant behavior under stress condition and for the development of new strategies and tools for enhancing plant growth in the future with a new scenario of climate change (Morales et al., 2024).

Furthermore, nitrogen availability is one of the main stressors in plants (Ciríaco da Silva et al., 2011). Nitrogen is an essential element that frequently limits plant growth in many terrestrial ecosystems (Vitousek, 1994). Recent studies suggest that responses to elevated [CO_2] depend on nitrogen form, and elevated [CO_2] reduces nitrate reduction, rizhosphere availability and inhibit nitrate assimilation in shoot of C_3 species, such as wheat and Arabidopsis, whereas ammonium utilization does not decrease, and exhibit greater stimulation from CO_2 enrichment (Rubio-Assensio and Bloom, 2017). Therefore, isotopic changes in the nitrogen cycle have been of great interest to plant physiology and ecology (Werner and Schmidt, 2002). In addition, the nitrogen status of a plant has a significant influence on its water relationship, as nitrogen and water often interact. When the soil experiences a prolonged period of drought, nitrogen mobility can be severely restricted. In fact, when a plant faces a water deficit, nitrogen deficiency occurs and rapidly inhibits plant growth (DaMatta et al., 2002). Whole-plant or organ nitrogen isotope compositions are determined by the isotope ratio of the external nitrogen source and physiological mechanisms within the plant. Whole-plant isotope composition can directly reflect that of the nitrogen source when the plant nitrogen demand exceeds the supply. Understanding the mechanisms underlying the control of whole plant, foliar nitrogen isotope composition, and source-sink ratio dynamics, will advance our knowledge of plant nitrogen acquisition and allocation, and how physiological transformations of nitrogen can influence whole-plant and leaf $\delta^{15}N$ (Evans, 2001). A better understanding of the interactive effects of [CO_2], water stress, and plant N is necessary to develop strategies to minimize the harm caused by drought and climate change.

The overall aim of this study was to explore the combined effects of different [CO_2] and water availability on C and N allocation, and their implications on the other plant processes. To date, the mechanisms conditioning the C and N allocation responses to different [CO_2] scenarios and the effects of different water conditions have not been sufficiently documented. Studying this may help us to understand the behavior of C_3 crop plants in future climate change scenarios. To better understand C and N partitioning among the organs of these plants exposed to depleted and elevated [CO_2], double labeling with $^{13}CO_2$ and $^{15}NH_4$–$^{15}NO_3$ was conducted.

This study tested several hypotheses. Long term exposure to depleted and elevated [CO_2] leads to physiological acclimation of wheat, but this physiological acclimation on wheat plants is modulated by water availability. Given that plants modify the biomass to different [CO_2], and water deficit induces reduction in biomass, it was hypothesized that the combinatory effect of both would lead to a large decrease in biomass in all the CO_2 treatments. The combined effect of both [CO_2] and water availability, leads to changes in C and N allocation on plants. During grain filling, the new sink (the spike) can modify physiological acclimation and the C and N balance between the source and sink. Given the higher stomatal closure under elevated [CO_2], it was hypothesized that plants under depleted [CO_2] would suffer more water deficit than plants in the future, that would be better adapted to drought.

Material and methods

Plant material

Durum wheat (*Triticum turgidum* var. Sula) was used in this experiment. Sula (released in 1994) is a wheat variety grown in Spain. It is characterised by its short stature, early heading, maturity, and high yield potential. Wheat seeds were germinated in petri dishes on wet Whatman paper. After 84 h, seedlings were transferred to 4-litre pots (one plant per pot) filled with quartz sand of 1 mm grain size.

Experimental design

Plants were grown in three fully controlled plant-growth chambers (Conviron E15, Controlled Environments Ltd., Winnipeg, Canada) at 22/18 ° C (day/night) and 60 % relative humidity. Plants were supplied with a photosynthetic photon flux density (PPFD) of 400 ± 30 mmol $m^{-2}s^{-1}$ during the 16 h light period (day). The humidity, temperature, and CO_2 levels in the chamber were continuously monitored by a sensor every 5 min (CMP3243 Controlled Environments Ltd, Winnipeg, Canada) and compared every two weeks with a Vaisala sensor (Vaisala MI70 Helsinki, Finland) with two indicators (HMP75 humidity and temperature; and GMP222; 0–2000 ppm carbon dioxide). Plants were watered with Hoagland complete nutrient solution (Arnon and Hoagland, 1939), alternating with distilled water in order to avoid salt accumulation over the whole life cycle.

Plants were grown during their entire life cycle (from September to January) under three different levels of CO_2 (700, 400 and 260 ppm) at the Experimental Fields Service of the University of Barcelona, Barcelona, Spain (more information is available in Aljazairi and Nogués, 2015). Forty-eight plants were placed in the first Conviron chamber and maintained at a high (future) CO_2 concentration level (ca. 731.7 ± 16.9 mmol mol^{-1}). Compressed industrial air was used to increase the CO_2 level in the chamber. The mix of the commercial CO_2 ($\delta^{13}C$ ca. −38.2 ‰) (provided by Carburos Metálicos S.A. Barcelona, Spain) with the ambient air ($\delta^{13}C$ ca. −12.5 ‰) resulted in a CO_2 isotopic composition of $\delta^{13}C$ ca. −22.6 ‰ inside the Conviron chamber. Another batch of forty-eight plants were placed in the second Conviron chamber, which was maintained at current CO_2 concentration levels (ca. 409.3 ± 2.5 mmol mol^{-1}). Finally, the same number of plants was placed in the third Conviron Chamber, which was maintained at pre-industrial CO_2 concentration levels (ca.259.4 ± 13.6 mmol mol^{-1}). In this third chamber, the CO_2 was reduced by pumping the air inside the chamber through a 1 L column filled with soda lime (Soda lime with indicator QP, Panreac Quimica SA., Barcelona, Spain). The soda lime was changed every two weeks. Plants were rotated inside the chamber every week and between the different chambers every three weeks to avoid chamber influences in the treatments.

Mild water-stress treatment (WS) was maintained by having the water content of soil pot capacity at 60 % applied to half of the plants in each plant-growing chamber. Contrary, control plants were well watered (WW) by maintaining 100 % water content of the soil pot capacity, which is the amount of water content held in the soil after excess water has drained away from the pot. Each pot was weighed every four days and refilled with water. Plant water status was evaluated by measuring the leaf relative water content (RWC, Weatherley, 1950).

Data measurements were obtained in three different plant stage periods (Pre-anthesis, T0; grain filling, T1; and the end of grain filling, T2).

Isotope labeling procedures with ^{13}C and ^{15}N

Simultaneous C and N labelling was conducted for the whole plant and at different CO_2 levels. For this reason, plants were placed inside Conviron growth chambers during the anthesis period and labelled with ^{13}C and ^{15}N. In addition, $\delta^{13}C$ was modified at elevated [CO_2] because of the $\delta^{13}C$ of the CO_2 was used to increase the [CO_2] within the growth chamber, according to Aljazairi et al. (2014b), Medina et al. (2016).

The $\delta^{13}C$ of air inside the three Conviron chambers was modified to distinguish it from $\delta^{13}C$ of ambient air. In the Conviron chambers, commercial CO_2 (AT% of ^{13}C ca. 99.9 % Carburos Metálicos SA, Barcelona, Spain provided by Eurisotop, Saint-Aubin, France) mixed with the ambient air ($\delta^{13}C$ ca. −12.5 ‰) resulted in a $^{13}CO_2$ isotopic composition of $\delta^{13}C$ ca. 165 ‰. Air samples were taken to analyze the CO_2 isotopic composition using gas-chromatography-combustion-isotope ratio mass spectrometry (GC/C/IRMS; Thermo Scientific™ GC IsoLink™) according to Nogués et al. (2004).

^{15}N labelling was also applied during the same period by replacing the ^{14}N in the Hoagland solution (Hoagland and Arnon, 1938) with double ^{15}N labeled ammonium nitrate ($^{15}NH_4$- $^{15}NO_3$, AT% of ^{15}N 10 %). After labelling, ^{15}N was removed by washing the quartz sand with distilled water. Plants were then irrigated with normal Hoagland solution. The double labelling was performed during anthesis so plants had the same amount of ^{13}C and ^{15}N available, regardless of the CO_2 or water treatment to which they were subjected.

C and N isotope compositions of total organic matter (TOM)

Samples from different parts of the plant (i.e. leaves, stems, roots and spikes) were dried in an oven at 60 ° C for 48 h and ground to a fine powder. Then, 1 mg was weighed in tin capsules, and the carbon and nitrogen isotope composition were determined using an Elemental Analyzer Flash 112 (Carbo Erba, Milan) coupled to an isotope ratio mass spectrometer (IRMS) Delta C Conflo III Interface (Termo Finnigan, Germany).

The results were expressed in conventional δ notation as parts per mil (‰). Carbon was referenced against the international standartV-PDB (Vienna Pee Dee Belemnite). Nitrogen was referenced using the international secondary standards with known $^{15}N/^{14}N$ ratios (IAEA N_1 and IAEA N_2 ammonium sulphate and IAEA NO_3 potassium nitrate) with reference to the international primary standard air N_2, which has a $\delta^{15}N$ value of 0 ‰.

Stable isotope compositions were expressed according to the following equation

$$\delta X = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}}\right) - 1 \tag{1}$$

where δ_X represents either $\delta^{13}C$, $\delta^{15}N$, and R denote the abundance of the ‘heavy’ to ‘light’ isotope ratio of samples and reference materials

Isotope discrimination (ΔX) was calculated from δ_a and δ_p (Farquhar et al., 1989) as:

$$\Delta X = \frac{\delta_a - \delta_p}{\delta_p + 1} \tag{2}$$

where ΔX represents either $\Delta^{13}C$, $\Delta^{15}N$, and a and p refer to air and plant for ^{13}C, and soil solution and plant for $\delta^{15}N$, respectively.

Open system for isotopic dark respiration determinations

The $\delta^{13}C$ of the respired CO_2 in dark conditions of the different plant organs (flag leaf, the remaining leaves, spikes, stems, and root) was studied in a respiration chamber previously described (Nogués et al., 2004). The chamber was connected in parallel to the sample air hose of a LI-COR 6400 system. The PPFD inside the chamber was maintained at 0 μmol m^{-2} s^{-1} by covering the chamber. The organ was first placed in the chamber with ambient air ($\delta^{13}C$ ca. −10.3 %). The chamber was then flushed with CO_2-free air and the CO_2 respired by the organ was collected using gas syringes (SGE International Pty Ltd, Australia) and stored in 10-mL vacutainers. The air in the three growing chambers (e.g. pre-industrial, current and future CO_2) was also sampled using 10-mL vacutainers. The CO_2 inside the vacutainers was analyzed using GC—C-IRMS.

All the GC—C-IRMS and EA/IRMS analyses were performed at the Scientific Technical Services of the University of Barcelona.

Proportion (p) of new C and N calculation

The proportion of ‘new’ carbon (derived from the labelling) in CO_2 respired in darkness after illumination and ‘new’ carbon and nitrogen in TOM were calculated according to Nogués et al., 2004:

$$x = 100\left(\frac{\delta Y' - \delta Y}{\delta o - AY - \delta Y}\right) \tag{3}$$

where $\delta Y'$ and δ_Y are the isotope compositions from the labelled and control, respectively; δ_o is the isotope composition of the outlet air and ΔY is the isotopic discrimination calculated using Eq. (4 for C for N).

$$\Delta^{15}N = \frac{\delta_a - \delta_p}{\delta_p + 1} \tag{4}$$

Leaf carbon and nitrogen content

Leaves used for gas exchange were collected, dried at 65 °C to a constant weight, and ground to a powder. Powder samples were assessed for the percentage C and N contents using an elemental analyzer at the Scientific Technical Services at the University of Barcelona, Spain.

Gas exchange analyses

An infrared gas analyzer (LI-COR 6400 system, LI-COR Inc., Lincoln, NB, USA) equipped with a Leaf Chamber Fluorometer (LI6400-40) was used to perform simultaneous measurements of gas exchange in an expanded flag leaf. A-Ci curves and dark respiration rates were conducted for each CO_2 level and water regimen.

The curves were repeated for four different plants per treatment and were measured from 0 to 2000 μmol mol^{-1} of CO_2. The light intensity was saturated at 1200 μmol photon m^{-2} s^{-1} of PPFD, at a temperature of 25° C. The parameters measured were: Assimilation Rate (A_{sat}), maximum CO_2 assimilation rates at saturated light (A_{max}), stomatal conductance (g_s) and Stomatal Limitation (SL) which were estimated at a PPFD of 1200 μmol m^{-2} s^{-1} using equations developed by Von Caemmerer and Farquhar (1981).

Growth parameters

Plant production in durum wheat under three CO_2 growth conditions (700, 400 and 260 ppm) and two water availability conditions were analyzed. Spike number per plant (SN); Spike weight per spike (SW/S, gr); Shoot weight (SW, gr) and Root weight (RW, gr). Further information is available in Aljazairi and Nogués (2015).

Data analysis

The CO_2 effects on wheat plant development were tested by two factors (CO_2 treatment and durum wheat genotype) analyses of variance (ANOVA). The statistical analysis was conducted using the SPSS 17.0 software package (SPSS Inc., Chicago, IL, USA). The means ± standard errors (SE) were calculated for each parameter. When a particular test was significant, the means were compared using Duncan's multiple comparison test. The results were considered statistically significant at $p < 0.05$.

Results

[CO_2] effects on plants

The different CO_2 treatments affected wheat plants isotopic composition. Before labelling (T_0), the $\delta^{13}C$ of TOM (natural abundance) was more ^{13}C enriched at depleted [CO_2] than at current [CO_2] (with increases of 4.1 ± 0.5 ‰ in spikes, 3.0 ± 0.2 ‰ in leaves, 3.8 ± 0.6 ‰ in stems, and 3.1 ± 0.4 ‰ in roots and at elevate [CO_2] was more ^{13}C depleted than at current [CO_2] (with decreases of 8.8 ± 0.1 ‰ in spikes, 8.1 ± 0.3 ‰ in leaves, 7.9 ± 0.4 ‰ in stems, and 9.3 ± 0.1 ‰ in roots) (Fig. 1).

During labelling, the $\delta^{13}C$ of the air in the three plant-growth chambers was ca. 165 ‰, and the $\delta^{13}C$ of TOM in labelled plants was more ^{13}C enriched than the corresponding non-labelled plants in all the CO_2 treatments (Fig. 1), where spikes were the main C sink. After labelling (T_1 and T_2), the $\delta^{13}C$ of TOM was more ^{13}C enriched at depleted than at current [CO_2] (with increases of 26.1 and 11.4 ‰ in spikes, 7.2 and 18.5 ‰ in leaves, 9.2 and 19.2 ‰ in stems, and 22.3 and 11.1 ‰ in roots; T_1 and T_2 respectively). At elevated [CO_2], ^{13}C was more depleted than at current [CO_2] (with decreases of 56.3 and 31.6 ‰ in spikes, 24.2 and 21.8 ‰ in leaves, 64.8 and 26.9 ‰ in stems, and 25.1 and 23.5 ‰ in roots; T_1 and T_2 respectively) (Fig. 1).

Before labelling (T_0), the $\delta^{15}N$ of TOM (natural abundance) showed no significant differences between depleted and current [CO_2]; however small increases in $\delta^{15}N$ were observed (with 0.1 ± 0. 3 ‰ in spikes, 0.8 ± 0.4 ‰ in leaves, 0.4 ± 3.4 ‰ in stems, and 2.3 ± 1.0 ‰ in roots). Also, no differences were found between elevated [CO_2] and current [CO_2]; however small increases in $\delta^{15}N$ were observed (3.7 ± 0.5 ‰ in spikes, 1.6 ± 0.3 ‰ in leaves, 0.8 ± 1.9 ‰ in stems, and 1.6 ± 0.7 ‰ in roots) (Fig. 1).

During labelling, the $\delta^{15}N$ value of the water solution in the three plant-growth chambers was ca. 4043.2 ‰. After labelling (T_1), the $\delta^{15}N$ of TOM in labelled plants was more ^{15}N enriched than the corresponding non-labelled plants in all the CO_2 treatments (Fig. 1) where spikes and roots were the main C sink. After labelling (T_1 and T_2), the $\delta^{15}N$ of TOM was more ^{13}C enriched at depleted than at current [CO_2] (with increases of 847.9 and 1007.4 ‰ in spikes, 410.0 and 978.6 ‰ in leaves, 983.7 and 1063.5 ‰ in stems, and 1076.8 and 323.7 ‰ in roots; T_1 and T_2 respectively). At elevated [CO_2], $\delta^{15}N$ was more enriched than at current [CO_2] (with increases of 105.0 and 462.1 ‰ in spikes, 758.9 and 548.2 ‰ in leaves, 935.9 and 122.7 ‰ in stems, and 548.4 and 501.3 ‰ in roots; T_1 and T_2 respectively), (Fig. 1).

Respired CO_2 is an important C sink for all organs, and we could observe significant differences between CO_2 concentration and ^{13}C of respired CO_2 in all the organs ($p < 0.005$ flag; $p < 0.0001$ spike; $p < 0.001$ stem; $p < 0.005$) except in leaf (F: 1.242; $p = 0.326$) (Table S2). Clearly, before labelling (T_0), plant respiration was less enriched in ^{13}C than after labelling (T_1 and T_2); however, respiration in spikes and stems was higher than that in flag leaves and other leaves. At T_0, respired CO_2 was more ^{13}C enriched at depleted [CO_2] than at current [CO_2], and the most depleted was under elevated [CO_2]. However, at T_1, respired CO_2 was more ^{13}C enriched at current [CO_2] than the other [CO_2]. We also observed that on the last sampling day (T_2), ^{13}C values of respired CO_2 were negative once again, and their values decreased when the [CO_2] increased (Fig. 2).

Fig. 3 shows the CO_2 effects on recently fixed C and N for wheat under two water treatments after ^{13}C and ^{15}N labelling. We assumed that 100 % of C or N supplied during short-term labelling could be assimilated by plants. If we observe the different plant organs, the percentage of new C was higher in spikes, followed by stems, roots, and leaves. However, the percentage of new N was higher in roots followed by stems and spikes and finally, the lowest values were founded in leaves. Fig. 3 also shows that the percentage of new C increases when the [CO_2] decreases. The same was true for the percentage of new N. However, at T_1, we observed that plants uptake more or similar percentages of new N at elevated [CO_2] than at current or depleted [CO_2].

Two different patterns were observed in% of new C and N under the [CO_2] effects (Fig. 3). The % of new C increased with increasing [CO_2]; however, the % of new N content decreased with increasing [CO_2]. The main sink for C and N content was root and stem, followed by leaf and spike. In general, the C and N increased between T_0 and T_1 and decreased at T_2, except the sink organs under elevated [CO_2] (Fig. 3).

Analyses of growth parameters showed that the [CO_2] treatments affected wheat plants. We found significant differences between [CO_2] treatments in terms of spike weight per spike (SW/S), shoot weight and root weight. Plants showed an increase in SW/S (i.e. 1.7; 2.0 and 2.5 mg; and 0.8; 1.3 and 2.5 mg, WW and WS respectively) and RootW (i.e. 8.8; 9.5 and 10.5 mg; and 3.1; 3.8 and 4.4 mg, WW and WS respectively) with increases in the [CO_2] (Table S1), but the opposite happened with the ShootW with decreases when the CO_2 increased. No significant differences were found between [CO_2] in spike number (SN, Table S1); but we observed a trend of decreasing SN when the CO_2 increased (more

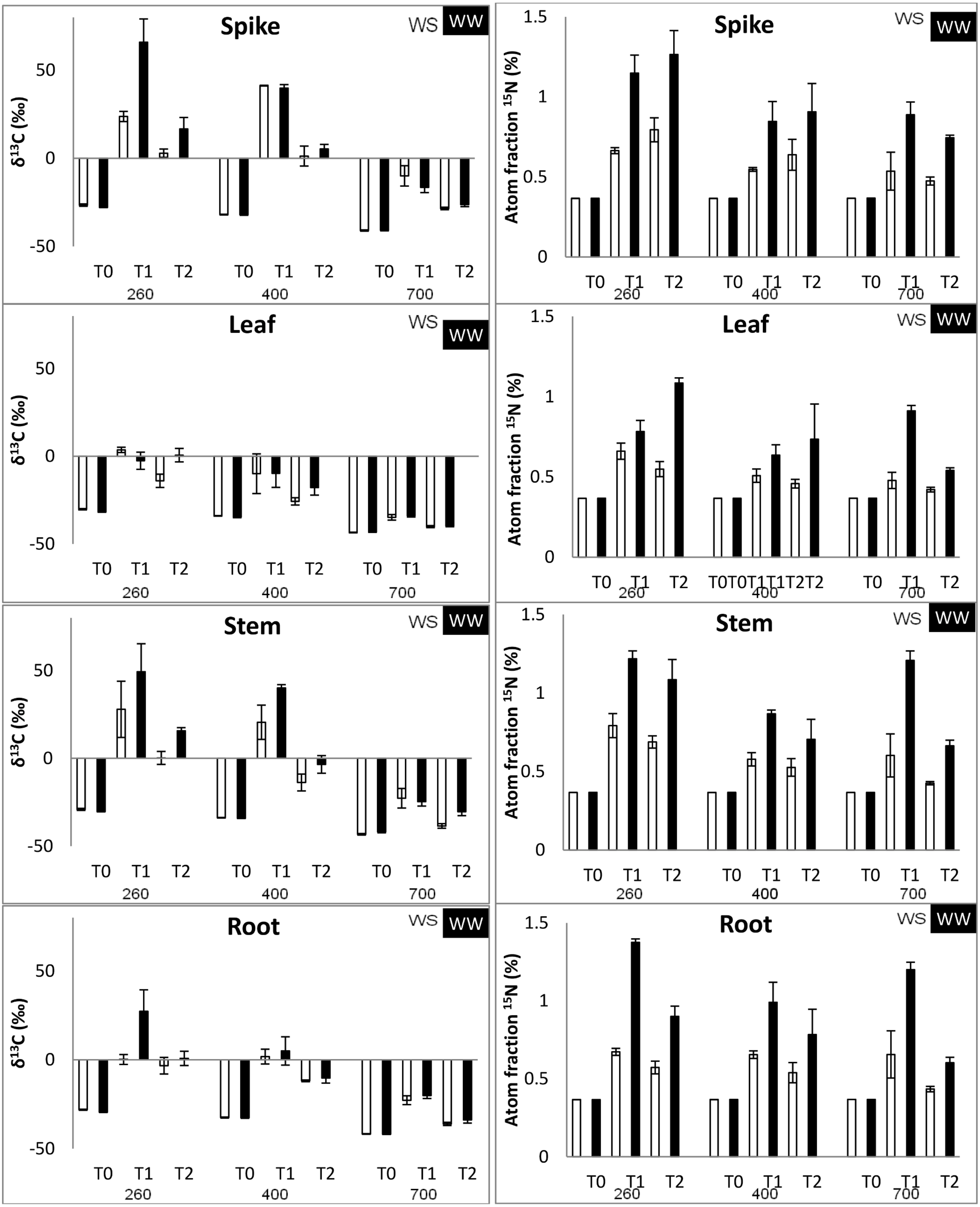

Fig. 1. CO_2 effects (700, 400 and 260 ppm) on $\delta^{13}C$ and $\delta^{15}N$ in TOM (flag, spikes, leaves, stems and roots) under two water treatments well watered (WW, close bars) and water stress (WS, open bars), before labeling (T0), 24 h after labeling (T1) and 7 days after labeling (T2).

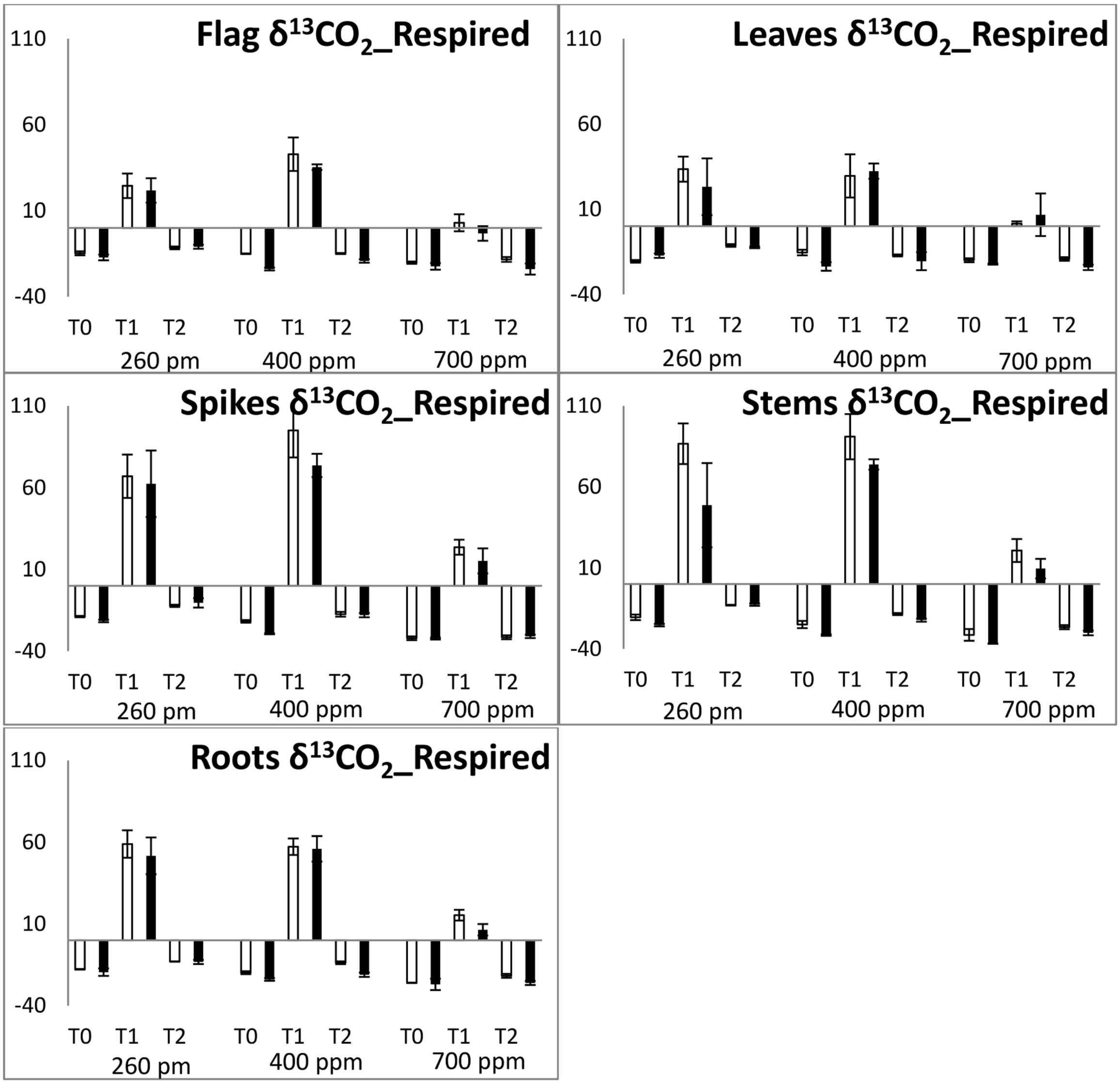

Fig. 2. CO_2 effects (700, 400 and 260 ppm) on $\delta^{13}CO_2$_Respired (flag, spikes, leaves, stems and roots) of wheat under two water treatments well watered (WW, close bars) and water stress (WS, open bars), before labeling (T0), 24 h after labeling (T1) and 7 days after labeling (T2).

growth parameters data are shown in Aljazairi and Nogués, 2015). However, under elevated [CO_2], plants showed less biomass than under current [CO_2] in terms of weight. A similar effect was observed in plants grown under depleted [CO_2].

Analyses of gas exchange parameters showed that the CO_2 treatments affected the plants. At elevated [CO_2], we observed that carboxylation activity was diminished or similar to the current [CO_2], as indicated by the reduction in A_{sat} and stomatal conductance. A_{sat} and stomatal limitation were similar or lower at depleted than at current [CO_2], but increased after prolonged exposure to depleted [CO_2], although differences between CO_2 treatments were not significant. (Table S2).

Water availability effects on plants

Analysis of stable isotopes also showed that the water treatments affected the plants under current [CO_2]. Before labelling (T_0), the $\delta^{13}C$ of TOM (natural abundance) showed no significant differences between WS and WW plants, but small increases in ^{13}C were observed in WS treatments. However, WW plants were more ^{13}C enriched than WS plants in all organs at T_1 and T_2 (with increases of 0.4 and 4.1 ‰ in spikes, 7.7 and 7.8 ‰ in leaves, 3.4 and 10.3 ‰ in stems, and 2.3 and 1.5 ‰ in roots at T_1 and T_2, respectively).

In addition, before labelling, the $\delta^{15}N$ of TOM (natural abundance) did not differ significantly between the WS and WW plants. However, WW plants were more ^{15}N enriched than WS plants in all organs at T_1 and T_2 (with increases of 594.2 and 751.5 ‰ in spikes, 786.7 and 772.9 ‰ in leaves, 621.1 and 499.2 ‰ in stems, and 1019.4 and 678.3 ‰ in roots, T_1 and T_2, respectively).

The ^{13}C of respired CO_2 ($\delta^{13}CO_2$_Respired) in the dark due to the different water availability (WW and WS) was observed with significant differences in flag ($p < 0.005$), spike ($p < 0.0001$), and stem ($p < 0.005$); but not in leaves (*F: 2.881*; $p = 0.118$) and root (*F: 2.724*; $p = 0.127$) (Table S2). In general, it was observed in all the organs that $\delta^{13}C$ losses through dark respiration were greater in WS than in WW treatments, before and after labelling, except in some leaves (Fig. 2).

The percentage of new C and N in TOM and two water treatments after short-term labelling (Fig. 3) revealed that the C stored was higher in WW plants than in WS plants for the different wheat organs. Between T_1 and T_2, the percentage of new C decreased, except in leaves WW plants under depleted [CO_2]. The percentage of new N was higher in the WW plants than in the WS plants for the different wheat organs. In general, between T_1 and T_2, the percentage of new N decreased, except in some leaves and spikes of WW plants.

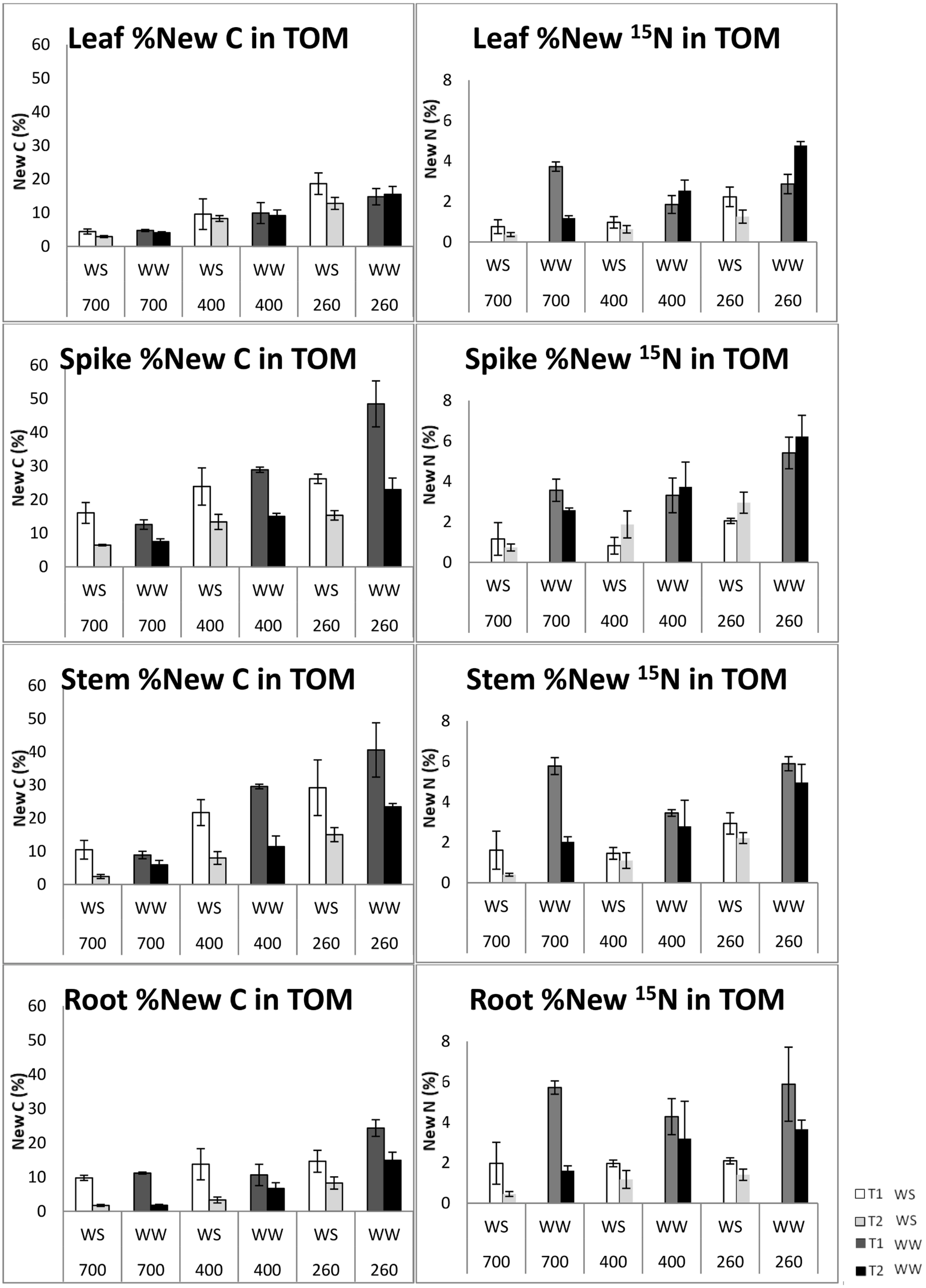

Fig. 3. CO_2 effects (700, 400 and 260 ppm) on %New C and %New N in TOM (spike, leaf, stem and root) of wheat under two water treatments well watered (WW) and water stress (WS), before labeling (T0), 24 h after labeling (T1) and 7 days after labeling (T2).

Analyses of gas exchange parameters showed effects of water treatment on plant assimilation and stomatal limitation (Table 1). In general, WW plants showed higher or similar assimilation and lower stomatal limitation than WS plants, some cases were modulated by [CO_2], as shown in the next section. However, we did not find significant differences in assimilation between water treatments, but in stomatal limitation was found.

[CO_2] effects on plants modulated by water availability

Analysis of stable isotopes also showed that the CO_2 effects are modulated by water availability on our plants, with significant differences between CO_2 and water treatments for ^{13}C (*$F = 8.352; p < 0.005; F = 6.037; p < 0.01; F = 4.013; p < 0.05$* for leaf, stem, and root respectively). However, no significant differences were found in spikes ($F = 2.041$; $P = 0{,}176$), but similar trends were observed in the other plant organs (Table S2). As we mentioned before, WW plants were more ^{13}C enriched than WS plants in all organs and CO_2 treatments. However, these differences were higher in depleted [CO_2] than at elevated [CO_2]. Plants were more ^{13}C depleted in elevated [CO_2] and WS than in current [CO_2] and WS treatments, and more ^{13}C enriched in the depleted [CO_2] and WW treatments than in the current [CO_2] and WW treatments. We also observed that plants were less ^{13}C enriched when CO_2 increased, but these differences were modulated by water treatments. Before labelling, these differences were not very large. But after, labelling (T_1), and overall, on the last sampling day (T_2), the differences were generally much higher at depleted [CO_2] and between water treatments as a sum of both effects (Fig. 1 and Table S2).

Analysis of ^{15}N showed no significant differences between CO_2 and water availability effects on our plants (*$F = 2.041; P = 0.176; F = 1.341; P = 0.301; F = 2.143; P = 0.164; F = 0.958; P = 0.414$* for spike, leaf, stem and root respectively); however, we observed similar trends and differences between treatments and plant organs (Table S2). Before labelling (T_0), the $\delta^{15}N$ of TOM was similar in all CO_2 and water treatments. However, during labelling, the $\delta^{15}N$ of the water solution in the three plant-growth chambers was ca. 4043,2 ‰ and after labelling (T_1 and T_2), the $\delta^{15}N$ of TOM in labelled plants was more ^{15}N enriched than the corresponding non-labelled plants in all the CO_2 and water treatments (Fig. 1). Spikes and roots were more enriched in ^{15}N; overall, at depleted CO_2 and WW treatments were the most enrichment than the other treatments. In addition, plants organs decreased $\delta^{15}N$ between T_1 and T_2, and this decrease was higher in WW plants, except for spikes and leaves of plants grown at elevated and current [CO_2] and WW treatments, and increased $\delta^{15}N$ as the main sink of ^{15}N (Fig. 1 and Table S2).

The ^{13}C of respired CO_2 ($\delta^{13}CO_2$_Respired) in the dark was also modulated by both parameters: [CO_2] and water availability. However, we observed significant differences only in spikes ($p < 0.005$; table S1). At T_0, we found that WS plants were more enriched in ^{13}C than WW plants, with higher differences between WS and WW plants in flag leaves, other leaves, and spikes, at current [CO_2] than the other CO_2 treatments. However, at T_1, the ^{13}C values of respired CO_2 values increased with decreasing CO_2 concentration, and we found higher differences between WW and WS plants at depleted than at current [CO_2]. Furthermore, the lowest ^{13}C of respired CO_2 differences between water treatments were found at elevated [CO_2]. Finally, we observed that on the last sampling day (T_2), ^{13}C values of respired CO_2 were negative once again and the differences between water treatments were small (Fig. 2).

The percentage of new C and N in TOM after short-term labelling revealed that the CO_2 effects were also modulated by water treatments. The percentage of new C was higher in WW than in WS plants and increased when [CO_2] decreased. In addition, leaves in depleted [CO_2] and WW plants showed an increased in the % of new C between T_1 and T_2 (Fig. 3). The % of new N increased when the [CO_2] decreased, and it was higher in WW than in WS plants at depleted, current, and elevated [CO_2] conditions for the different plant organs. This difference was very high between water treatments at both times. During T_1, the % of new N increased but decreased during T_2. However, the spike had % increased of N at current and depleted [CO_2] in both water treatments and became as the main sink of N during the grain filling. It also happened in WW leaves (Fig. 3).

The C and N contents (mg/mg) were affected by [CO_2] and these effects were modulated by water availability in plants (Fig. 3). The C content was higher in WW than in WS plants and increased as the [CO_2] increased. Higher differences were observed between water treatments under elevated [CO_2] than at depleted [CO_2]. Plants showed higher C content under elevated [CO_2] (i.e. 5 % and 3 % under WW and WS respectively) and lower leaf C content under depleted [CO_2] (i.e. 3 % and 5 % under WW and WS respectively) than current [CO_2], but the differences were not significant. However, the N content was higher in WW than in WS plants at depleted and current [CO_2] conditions, but it was lower in WW than in WS plants under elevated [CO_2] conditions. At depleted and elevated [CO_2], plants had a lower N content, with a larger difference at elevated [CO_2] (48 % and 6 % less N for WW and WS, respectively) than at the current [CO_2], whereas at depleted [CO_2], plants also had a higher N content (16 % and 4 % for WW and WS, respectively) (Fig. 3).

Analyses of growth parameters showed that [CO_2] effects were modulated by water treatments on wheat plants. WW plants showed more down-regulation of growth under elevated [CO_2] than WS plants, with decreases across a range of growth parameters (Table 1; Table S1).

Analyses of gas exchange parameters showed that the [CO_2] effects were modulated by water treatments. At current [CO_2], WW conditions resulted in higher assimilation and lower stomatal limitation than mild WS conditions. At depleted [CO_2], lower values in both, assimilation and stomatal limitation, were found compared to current [CO_2]; however, some of the parameters of assimilation increased during grain filling, thus indicating up-regulation of photosynthesis. At elevated [CO_2], WW conditions resulted in lower assimilation and lower stomatal limitation than mild WS because of photosynthetic acclimation. However, we did not find any significant differences between [CO_2] x water treatments (Table S2).

Discussion

One of the main goals of this study was to analyse the combined effects of three different [CO_2] (i.e. depleted, current and elevated) under mild water stress on C and N allocation in durum wheat plants

Table 1

CO_2 effects (260, 400 and 700 ppm) on Biomass (Number of spike per plant, NS; weight of spike/spike (grams), WS/S; weight of shoot (grams), WShoot and weight of root (grams), WRoot), Photosynthetic Assimilation at saturation light rate (A_{sat}) and Stomatal limitation (SL) of wheat, under two water treatments well-watered (WW) and mild water-stress (WS).

CO_2	Water	NS	WS/S (gr)	WShoot (gr)	WRoot (gr)	A_{sat}	SL
700	**WW**	2,3Aa ± 0,3	2.5Aa ± 0,1	16,0Ba ± 0,9	10,5 Aa ± 1,9	15,17Aa ± 0,8	22.7 Aa ± 3.5
700	**WS**	1,3Ab ± 0,3	2.5Aa ± 0,3	6,5Aa ± 1,2	4,4 Ab ± 0,2	16,57Aa ± 1,9	30.5Ab ± 4.0
400	**WW**	3,7Aa ± 0,7	2Aa ± 0,3	24,8Aa ± 1,7	9,5 Aa ± 1,2	18,03Aa ± 1,8	20.2 Aa 8 ± 3.3
400	**WS**	2,7Ab ± 0,3	1.3Bb ± 0,2	8,2Ab ± 1,8	3,8 Ab ± 1,1	15,67Aa ± 3,8	28.8Ab ± 5.6
260	**WW**	2,7Aa ± 0,7	1.7 Ba ± 0,7	19,0 Ba ± 2,0	8,8 Ba ± 1,3	18,27Aa ± 1,3	20.2 Aa ± 1.4
260	**WS**	2,7Aa ± 0,3	0.8 Ba ± 0,3	10,3 Aa ± 3,7	3,1 Aa ± 0,3	4,67Ba ± 1,27	15.1Bb ± 5.0

(*Triticum turgidum)*. This study showed that CO_2 treatments affected the plants, and that these effects were modulated by water availability.

[CO_2] effects on plants

The mechanism of conditioning the C and N allocation responses to different [CO_2] has been described in previous studies. Aljazairi et al. (2014b) and Nogués and Azcón-Bieto (2013), showed that wheat plants can acclimatize their photosynthesis rate to the long-term exposure to depleted and elevated [CO_2] conditions. Photosynthetic acclimation is the physiological adjustment of plants to a given [CO_2]. But this acclimation can be conditioned by other factors such as plant phenology, other stresses (i.e. water stress), and environmental conditions (Amthor, 2001; Nowak et al., 2004; Leakey et al., 2009; Alshaal et al., 2017; Pardo et al., 2009; Aranjuelo et al., 2009b, 2011a; Aljazairi and Nogués, 2015; Zhang et al., 2024).

For depleted [CO_2] and during tillering, similar values of gas exchange parameters (i.e. A_{sat}) were found compared to current [CO_2]. During grain filling, those values (i.e. A_{sat}) increased, showing up-regulation of photosynthesis. This increase in assimilation during grain filling under depleted [CO_2] resulted in increased of C and N content, % of new C and N or higher labelling of ^{13}C and ^{15}N than current [CO_2] (Aranjuelo et al., 2011b) because, at depleted [CO_2], wheat had C source limitations, as we can observe before the labelling. Plants showed higher allocation of C to green areas (photosynthetically active) than roots, compared to the other CO_2 treatments (Aljazairi and Nogués, 2015; Aranjuelo et al., 2011a). However, this increase in C allocation on green areas did not result in an increase in biomass unlike plants under current [CO_2] conditions (Aljazairi and Nogués, 2015), as shown in other studies (Allen et al., 1991; Dippery et al., 1995; Gerhart and Ward, 2010; Aranjuelo et al., 2011). This suggests that in the past, plant species reduced the potential productivity, and for that, the C uptake from the atmosphere.

Lower values of gas exchange parameter (i.e. $A_{sat,}$) was found, indicating down-regulation of photosynthesis at elevated [CO_2] in comparison to current [CO_2] condition. Sage and Coleman (2001) demonstrated that plant acclimation processes during long term exposure to different [CO_2] can compensate for the effects of CO_2 variation on plant processes. This implies that significant photosynthetic adjustments may have taken place in the past and will occur in the future, thereby changing the allocation and balance of C and N in plants. However, during grain filling, there was an increase in photosynthetic assimilation, breaking the down regulation, as shown in Aljazairi et al. (2014a).

Plant growth was also modulated by the different [CO_2], which implies that significant adjustments in C and N allocation have been made (Fuertes-Mendizabal et al., 2010; Reddy et al., 2010). Plants can modify photosynthetic assimilation, the biomass and the C and N to be send to the different sinks. As suggested by Urban (2003), one of the parameters that can affect photosynthetic regulation by [CO_2] is the modification of the source-sink ratio. Aranjuelo et al. (2013), found that the plant capacity to increase the biomass was lower at current or depleted [CO_2] than at elevated [CO_2]. Moreover, spikes, roots and stems were the main C and N sinks, especially under elevated [CO_2]. Plant growth under depleted [CO_2] can be associated with increased C allocation to the shoot (spike and stem). These modifications of the source-sink ratio by depleted [CO_2] has often been observed in previous studies (Gebauer et al., 1996; Poorter and Nagel, 2000; Lehmeier et al., 2005; Aljazairi et al., 2014b), and resulted from direct responses of allocation to stress or disturbance, that favoured the plant part which intercepts the most severely growth limiting resource. In our case, at depleted [CO_2], plants favoured shoot growth to assimilate as much C as possible and may have stimulated the allocation of C towards leaves and shoots because of C necessity. However, at elevated [CO_2], most studies show an initial enhancement on growth and photosynthesis, but this enhancement disappears in time (Bazzaz, 1990; Slot et al., 2021). Plants can increase C allocation in roots because the C source is very high (Lawlor and Mitchell, 1991; McConnaughay and Coleman, 1999; Poorter and Nagel, 2000). It is also associated to decreases in leaf or spike biomass, decreases in N content and Rubisco activity (Thompson et al., 2017; Gojon et al.; 2023) and increases in starch levels (Yilmaz et al., 2017). Increased biomass allocation to the roots has been shown to be caused by the N-limitation on leaf at elevated [CO_2] (Soussana et al., 1996).

Another example is that wheat has a high requirement for N during grain formation. Grain nitrogen content is taken from N that is remobilized from different parts of the plant (stored before grain filling) or new uptake nitrogen in that same period (Dupont and Altenbach, 2003). Like in our case, the nitrogen content in all the organs under elevated [CO_2] is less than that under depleted [CO_2] (Mitsutoshi et al., 2005). Elevated CO_2 may reduce the N content of plant tissues. Consequently, elevated CO_2 may drive N deficiency into the plant (Tuba and Lichtenthaler, 2007). However, the mechanisms controlling N and C allocation are still not well understood (Farrar and Jones, 2000; Gojon et al., 2023), because the relationship between C and N input and metabolism are made complicated by dynamic exchanges between plant organs, and atmospheric [CO_2] has a strong impact on the physiology of plants and a direct effect of nitrogen uptake and assimilation (Gojon et al., 2023).

The allocation and partitioning of C and N among plants and their organs can be studied using stable isotopes as tracers. Understanding the mechanisms controlling whole wheat plant N and C isotope composition will further advance our knowledge of plant N and C acquisition and allocation in different climatic scenarios. For this reason, we performed $^{15}N/^{14}N$ and $^{13}C/^{14}C$ labelling during the grain filling because of the importance of this period in the C and N allocation in wheat plant (Fig. 1). Fig. 3 shows the CO_2 effects on recently fixed C and N for wheat under two water treatments after labelling. We assumed that 100 % of C or N supplied during short-term labelling could be assimilated by the different parts of the plant, thereby permitting us to calculate the proportion of “new” C and N (i.e. recently fixed or uptake) in TOM and respired CO_2 (Nogués et al., 2004; Ghashghaie and Badeck, 2013). This C is allocated through three main processes: **i)** storage in the plant tissues (in our case the ^{13}C and % of new C increased when the [CO_2] decreased (Figs. 1 and 3), **ii)** translocation to other organs of the plant (in our case plants sent more ^{13}C and % of new C to green organs at depleted than elevated [CO_2] (Figs. 1 and 3), and **iii)** losses through plant respiration (in our case, lower losses of ^{13}C were observed at elevated [CO_2], and these losses were higher in spike and stem organs than in leaves or roots (Fig. 2) (Ghashghaie and Badeck, 2013; Schnyder et al., 2003; Nogués et al., 2004).

The study of ^{15}N in plant N cycle under different [CO_2] is a powerful tool to assess whether processes, in the N cycle, are influenced by rising atmospheric [CO_2] (Robinson, 2001; Gojon et al., 2023) and could guide future studies to identify the exact processes involved in the N cycle response to future climate change (Bassirirad et al., 2003). In our case, we found out that [CO_2] strongly influenced the patterns of assimilation and allocation of N in plant organs (as in Yoneyama and Kaneko, 1989; Yoneyama et al., 1991; Evans et al., 1996; Halpem et al., 2022). Moreover, we observed that the uptake, transport, accumulation, reallocation, and excretion of nitrogen during growth caused differences in concentrations among organs (i.e. between leave and spike or stem) because most reactions discriminate against ^{15}N (Shearer and Kohl, 1986; Yoneyama et al., 1998). Also, differences are due to phenology and source-sink ratio (increases of ^{15}N and N content during grain filling in all organs, overall in spike and stem; Aranjuelo et al., 2012); and because of [CO_2] treatments (higher ^{15}N and N content at depleted than elevated [CO_2]; Aljazairi and Nogués, 2015; Halpem et al., 2022; Gojon et al., 2023).

Water availability effects on plants

Well-watered (WW) and mild-water stress (WS) treatments showed significant changes in the response to C and N uptake. Many studies have identified the water regimen as one of the main factors of global change that controls plant productivity and C and N allocation (Rao et al., 2016). Our data suggest that WW plants have a large capacity to create new sinks during grain filling and can accumulate greater quantities of C, and especially N. These reserves are mainly sent to spikes, but also to roots and stems (Ward and Strain, 1997). In our study, C and N allocation during grain filling was conditioned by different factors: (i) the availability of these elements to plants (Fuertes Mendizabal et al., 2012), (ii) the water availability (Aranjuelo et al., 2007) and (iii) CO_2 availability. Nogués and Baker (2000) showed that drought induces lower photosynthetic capacity suggesting that drought decreases Rubisco capacity. But also, transcription profiles under water stress suggested an inhibition of primary C fixation and N assimilation (Medina et al., 2016). As we previously reported (Aljazairi and Nogués, 2015), WS reduced the water status, and consequently the photosynthesis, the plant growth, and the C uptake were also reduced. Drought also inhibited the N uptake through their effect on biomass production and plant could suffer down expression of Rubisco, chlorophyll, and other pigments to diminish damage by ROS production (Aranjuelo et al., 2007; Dalal and Tripathy, 2012). Ciriaco da Silva et al. (2011) showed that under drought stress, there is a reduction in nutrient uptake by the roots partially due to the reduction in soil moisture, which causes a decreased rate of N diffusion from the soil to the absorbing root surface and translocation to the leaves and then to the reproductive organs like in our case. In other words, drought causes low nitrogen availability in the soil and lower nutrient transport in plants (Hu et al., 2007). The percentage of new C in TOM and $\delta^{13}C\_CO_2$ respired during the grain filling revealed that the C stored was higher in WW than in WS plants for the different wheat organs and that the spike and Stem were the organs with the highest percentage of new C and N.

[CO_2] effects on plants are modulated by water availability

Our experiment showed significant changes in C and N uptake and plant growth under both, CO_2 (depleted, current, and elevated [CO_2]) and water availability (WW and WS) factors. The main global change factors controlling plant productivity are CO_2, temperature, water regimes and Nitrogen deposition (Alshaal et al., 2017).

In addition, evaporative water loss from leaves during the CO_2 uptake is unavoidable. Therefore, when water is a limiting factor, it is advantageous for plants to increase their water use efficiency. A fundamental component of plant productivity response is the control of stomatal conductance to CO_2 and water vapour. Many studies on plants grown under elevated and depleted [CO_2] have shown the relationship between CO_2 assimilation rate and the conductance at which plants grow (Franks and Hoffmann, 2012; Li et al., 2017). Following the initial CO_2 change from the environment, stomatal conductance seemingly adjusts back towards a value that optimises carbon gain with respect to water loss (Cowan and Farquhar, 1977; Dubbe et al., 1978; Farquhar et al., 1978; Medlyn et al., 2011). This involves reducing stomatal conductance (increasing stomatal limitation) under elevated [CO_2] (Gojon et al., 2023) or increasing stomatal conductance (decreasing stomatal limitation) under depleted [CO_2] (Batke et al., 2020). This was observed in the plants and is widely observed in other studies (Ainsworth and Rogers, 2007; Aljazairi et al., 2014a; Zhang et al., 2022). Water stress in plants reduced transpiration by closing stomata; therefore, there is a substantial reduction of net CO_2 assimilation. Besides, depleted [CO_2] worsened CO_2 assimilation rate, while elevated [CO_2] enhanced CO_2 assimilation rate in wheat plants. Our plants showed photosynthetic upregulation at depleted [CO_2] and photosynthetic downregulation at elevated [CO_2], but it was broken during the grain filling, the same trend was observed by Aljazairi et al. (2014a). However, plants grown under mild WS increased the stomatal limitation to avoid water loss. But under depleted [CO_2], WS plants suffered more than in all the other treatments and decreased both the stomatal conductance and the photosynthesis assimilation. This indicates that our plants were less resistant to drought at depleted [CO_2], a possible scenario in the past when the [CO_2] was lower.

Plants grown under depleted [CO_2] exhibited lower C assimilation, and those grown under elevated [CO_2] exhibited higher C assimilation, relative to plants growing at current [CO_2] (Franks and Hoffmann, 2012). As in our case, at depleted [CO_2], lower or similar A_{sat} values were found in comparison to current [CO_2]. During grain filling, A_{sat} values increased under depleted [CO_2] (ca. 26.13 ± 1.95 and 22.90 ± 0.7 in WW and WS, respectively), showing up-regulation of photosynthesis. The consequence was not only a net C and N content increase, but also increase in % of new C and N, or higher labelling of ^{13}C and ^{15}N compared to current [CO_2], especially in WW plants. However, we must consider that during the labelling, the availability of C and N is very high, and plants grown under depleted [CO_2] are more receptive for this new C and N than plants grown under elevated [CO_2], as published by Aljazairi et al., (2014). Other studies showed that drought reduced leaf growth by inhibiting cell division and expansion as well as plant growth, as we showed in Aljazairi and Nogués (2015), where the growth of plant organs was lower under mild-WS than WW treatments. However, elevated [CO_2] and drought have opposite interacted effects on growth, since elevated [CO_2] favours growth while WS reduces growth. In our case, the difference in plant biomass between watering regimes was also lower at elevated [CO_2], but this difference was not significant as reported in other CO_2 studies (AbdElgawad et al., 2015; Gámez et al., 2023; Tcherkez, et al., 2020). Elevated [CO_2] in combination with mild-WS, the biomass is similar or greater to that of WW plants (Ottman et al., 2001; Kimball et al., 2001; Varga et al., 2017). This shows that the [CO_2] effects are modulated by the water availability, and, as we mentioned before, there is an interactive effect between elevated [CO_2], N and water regimen (Ofori-Amanfo et al., 2023). The effects of depleted and elevated atmospheric [CO_2] on plants under drought are complex.

The photosynthetic effect of depleted [CO_2] can be worse under drought conditions because of the stomatal opening and the water loss. In contrast, under elevated [CO_2] and WS, the photosynthesis is stimulated by the CO_2 availability, even though the stomatal closure and consequently, water stress is lesser than in depleted [CO_2] (Robredo et al., 2007). These data suggest that the relative photosynthetic benefits of elevated [CO_2] are greater in more arid regions due, at least in part, to water savings through stomatal closure (Morgan et al., 2004).

Besides, prolonged suppression of CO_2 assimilation under prolonged drought may require plants to utilise stored carbohydrates to maintain metabolism, thereby enhancing the chances of survival (McDowell et al., 2011). We found that our wheat at WS decreased the C and N uptake compared to WW plants, and the growth was inhibited, especially under depleted [CO_2]. Drought may also overwhelm the benefits of elevated [CO_2] (Kimball et al., 2001; Sneed, 2018). In our study, the plants' growth under elevated [CO_2] was similar to that of current [CO_2] because of photosynthesis acclimation. However, this growth was lower under WS, indicating that drought has counteracted and modified the effects of CO_2 (Gedalof and Berg, 2010; Phillips et al., 2009; West et al., 2005).

As mentioned before, the C and N allocation in the plant depends on the different environments, such as increased CO_2 (Aljazairi et al., 2014a), water availability (Ahmadi and Baker, 2001; Aljazairi and Nogués, 2015) or N nutrition (Gojon et al., 2023), but also depends on the source-sink ratio that clearly changes during grain filling (Asseng and Van Herwaarden, 2003; Aranjuelo et al., 2012; Barzegar et al., 2013). These factors interact simultaneously and modulate the response and the direction of the C and N allocation. However, very little is known about the mechanisms that control the C and N allocation between the grain, new photoassimilates and reserve pools (Schnyder, 1993; Lemoine et al., 2013; Martínez-Peña et al., 2022). With the stable isotopes we could observe and follow the different origins and allocations of C and N under CO_2 and water treatments during grain filling. Our data suggests that during grain filling and at depleted [CO_2], plants were more receptive and conservative (for example, lower losses by respiration of C) for C and N than at elevated [CO_2]. However, water stress reduces this C and N uptake capacity. Under depleted [CO_2] and WW conditions, most of these new photoassimilates were sent to reproductive organs. WS treatment also affected the N uptake during grain filling, showing lower ^{15}N uptake capacity from soil in all the CO_2 treatments, especially under depleted [CO_2]. Moreover, under depleted [CO_2], the ^{15}N losses were lower during the grain filling, even increasing together with the % of new N at the end of the grain filling in spikes and leaves. This effect was higher in WW than in WS plants, suggesting that N is mobilized from storage plant areas (stems and roots) to the grain, because of the low availability of CO_2 in the environment. For this reason, photoassimilates (i.e. sugars) and reserves (i.e. starch) play an important role during metabolite transport for grain filling and storage carried out by plant organs (Lemoine et al., 2013), and facilitate whole plant communication, particularly in the transmission of information concerning the resources availability (Griffiths et al., 2016). The relationship between C and N inputs and metabolism are further complicated by dynamic exchanges between plant organs and the effect of the environment (Baghalian et al., 2014).

Conclusions

Using long-term treatments with different CO_2 concentrations (depleted, current, and elevated) and water availability (well-watered and mild water stress), as well as short term labelling with ^{13}C and ^{15}N in wheat durum plants, we found the following:

- The plants' response to different environmental [CO_2] was modulated by water availability, and therefore, C and N allocation was also modulated by both factors.
- The physiological plant response was acclimation to different environmental CO_2 was modulated by water availability; the photosynthetic acclimation affected the C and N allocation in plants. However, this acclimation was broken during grain filling changing the C and N allocation in Durum wheat plants (*Triticum turgidum* var. Sula).
- Plants under depleted [CO_2] presented lower drought tolerance than those under the current [CO_2]. Plants under elevated [CO_2] presented higher drought resistance than those in the current [CO_2].

However, the mechanisms controlling whole C and N plant acquisition and allocation under different climatic scenarios with environmental stresses in wheat plants are not completely clear and require further studies.

CRediT authorship contribution statement

Salvador Aljazairi: Writing – original draft, Methodology, Investigation, Conceptualization. **Brigen Manikan:** Writing – original draft, Conceptualization. **Xavier Serrat:** Writing – original draft, Investigation, Conceptualization. **Salvador Nogués:** Writing – original draft, Supervision, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Salvador Nogues reports financial support was provided by University of Barcelona.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.stress.2024.100663.

Data availability

No data was used for the research described in the article.

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