Differential Flag Leaf and Ear Photosynthetic Performance Under Elevated (CO$_2$) Conditions During Grain Filling Period in Durum Wheat

Angie L. Gámez$^1$, Rubén Vicente$^{2,3}$, Rut Sanchez-Bragado$^4$, Iván Jauregui$^5$, Rosa Morcuende$^3$, Nieves Goicoechea$^6$ and Iker Aranjuelo$^{1,*}$

$^1$Instituto de Agrobiotecnología, CSIC-Gobierno de Navarra, Mutitua, Spain, $^2$Instituto de Tecnología Química e Biológica António Xavier, Universidade NOVA de Lisboa, Oeiras, Portugal, $^3$Institute of Natural Resources and Agrobiology of Salamanca, IRNASA-CSIC, Salamanca, Spain, $^4$Department of Crop and Forest Sciences, University of Lleida – AGROTECNIO Center, Lleida, Spain, $^5$Plant Genetics, TERRA Teaching and Research Center, University of Liège, Gembloux, Belgium, $^6$Departamento Biología Ambiental, Grupo de Fisiología del Estrés en Plantas, Facultad de Ciencias (Unidad Asociada al CSIC, EEAD, Zaragoza, e ICSV, Logroño), Universidad de Navarra, Pamplona, Spain

Elevated concentrations of CO$_2$ (CO$_2$) in plants with C$_3$ photosynthesis metabolism, such as wheat, stimulate photosynthetic rates. However, photosynthesis tends to decrease as a function of exposure to high (CO$_2$) due to down-regulation of the photosynthetic machinery, and this phenomenon is defined as photosynthetic acclimation. Considerable efforts are currently done to determine the effect of photosynthetic tissues, such us spike, in grain filling. There is good evidence that the contribution of ears to grain filling may be important not only under good agronomic conditions but also under high (CO$_2$). The main objective of this study was to compare photoassimilate production and energy metabolism between flag leaves and glumes as part of ears of wheat (Triticum turgidum L. subsp. durum cv. Amilcar) plants exposed to ambient [$a$(CO$_2$)] and elevated [$e$(CO$_2$)] (400 and 700 µmol mol$^{-1}$, respectively). Elevated CO$_2$ had a differential effect on the responses of flag leaves and ears. The ears showed higher gross photosynthesis and respiration rates compared to the flag leaves. The higher ear carbohydrate content and respiration rates contribute to increase the grain dry mass. Our results support the concept that acclimation of photosynthesis to e(CO$_2$) is driven by sugar accumulation, reduction in N concentrations and repression of genes related to photosynthesis, glycolysis and the tricarboxylic acid cycle, and that these were more marked in glumes than leaves. Further, important differences are described on responsiveness of flag leaves and ears to e(CO$_2$) on genes linked with carbon and nitrogen metabolism. These findings provide information about the impact of e(CO$_2$) on ear development during the grain filling stage and are significant for understanding the effects of increasing (CO$_2$) on crop yield.

Keywords: ear physiology, elevated CO$_2$, durum wheat, photosynthesis acclimation, gene expression
INTRODUCTION

Atmospheric CO$_2$ concentrations (CO$_2$) have increased considerably since the pre-industrial era ($\approx$280 ppm), reaching 414.5 ppm by 2020 (Noaa/Esrl, 2020). Estimated models for future greenhouse scenarios predict that CO$_2$ concentration will not stabilize but will continue increasing over the coming decades (Ipcc, 2020). High (CO$_2$) in plants with C$_3$ photosynthetic metabolism, such as wheat, stimulate photosynthetic rates and consequently an increase in plant biomass is observed despite decreases in stomatal conductance (Aranjuelo et al., 2011). However, this response is not linear, and after the first stimulation, the photosynthetic rate tends to decrease as a function of the duration of high (CO$_2$) exposure, which is due mainly to accumulation of sugars and down-regulation of photosynthetic machinery, including a decrease in Rubisco protein content and activity. This phenomenon is named photosynthetic acclimation (Aranjuelo et al., 2011; Dusenge et al., 2019). Leaf photosynthesis benefits from elevated CO$_2$ by stimulating carboxylation of Rubisco, which is generally substrate limited (Farquhar et al., 1989), and by inhibiting the Rubisco oxygenation reaction, which leads to a loss of carbon (C) fixed during C$_3$ photosynthesis (Long et al., 2004). The increases in leaf photosynthesis generate a remarkably enhanced production of different carbohydrates, which alters downstream processes (Ainsworth and Rogers, 2007). Moreover, low sink strength capacity appears to be related to photosynthetic down-regulation (Aranjuelo et al., 2011). Previous studies (Paul and Foyer, 2001; Borrell et al., 2015; Griffiths et al., 2016) have demonstrated that sink strength limits leaf photosynthesis. Thus, further work in understanding leaf and ear photosynthesis and C allocation is required to evaluate the ability of crops to exploit projected increases in atmospheric (CO$_2$).

The ears are composed by awns, glumes, and ear bracts (lemma and paleae). During the last decade, significant effort has been put into understanding the photosynthetic contribution of entire ears and individual organs from ears, i.e., the glume, to grain filling in crops subjected to different growth conditions (Sanchez-Bragado et al., 2020 and references included). Recent evidence indicates that the contribution of ear photosynthesis to grain filling may be important not only under drought but also under good agronomic conditions (Maydup et al., 2010; Sanchez-Bragado et al., 2014a,b; Sanchez-Bragado et al., 2016). Although it has not been clearly demonstrated that ear photosynthetic rate under good agronomic conditions is higher than in the flag leaf lamina (Xu et al., 2009), a significant proportion of the C accumulated in wheat grains may originate in the ears (Aranjuelo et al., 2011; Sanchez-Bragado et al., 2014b; Zhou et al., 2014). In fact, ears exhibit better performance not only under water stress (Tambussi et al., 2007; Vicente et al., 2018) but also under high CO$_2$ environments compared with the flag leaf, with the ear contributing more to grain yield (Zhu et al., 2008). Nevertheless, the net photosynthesis of the ear per unit one-sided organ area is lower than in the flag leaf lamina due to the presence of larger amounts of heterotrophic tissues in the ear. The capacity of ears to re-fix respired CO$_2$ is twice the capacity of the flag leaf lamina (Araus et al., 1993; Bort et al., 1996; Gebbing and Schnyder, 2001; Tambussi et al., 2007, 2005; Vicente et al., 2018). Further, the xeromorphic anatomy of the ears (sclerophyllous traits of the bracts and particularly the awns) combined with a higher water use efficiency (Araus et al., 1993) and a greater osmotic adjustment (Tambussi et al., 2007, 2005) confer on the ears a higher relative water content compared to the rest of the plant (Morgan, 1980; Blum et al., 1988). Consequently, this mechanism to avoid dehydration in the ear results in delayed drought-induced or heat-induced senescence relative to the flag leaf lamina (Tambussi et al., 2007; Vicente et al., 2018). In addition, a longer developmental duration under high (CO$_2$) environments has been observed in the ear compared to the flag leaf (Zhu et al., 2008).

A number of studies have revealed the important role of ears under different control and stressful growth conditions (Abebe et al., 2010; Jia et al., 2015; Sanchez-Bragado et al., 2014b, 2016, 2020). Although higher (CO$_2$) are required to saturate photosynthesis in ears compared to the flag leaf in wheat (Knopf et al., 1986), ear photosynthesis under elevated CO$_2$ has been widely underestimated in the past (Teramura et al., 1990; Andre and Ducloix, 1993). Therefore, recent studies have sought to estimate the role of ears under elevated CO$_2$ by deploying Free-Air CO$_2$ Enrichment (FACE) (Weschung et al., 2000; Zhu et al., 2008, 2009) and semi-controlled conditions (Li et al., 2019). In addition, molecular markers associated with morphological traits of the ear have been identified in wheat (Sourdille et al., 2002; Molero et al., 2019) and assessment of the key metabolic profiles of ear bracts has also been undertaken in a non-destructive manner using spectroradiometers (Vergara-Diaz et al., 2020). However, until recently, studies to estimate the photosynthetic performance of the ear that have combined analyses at the physiological, metabolomic, and transcriptomic level have been scarce, especially in high CO$_2$ environments. Nonetheless, evidence from integrative analysis of the transcriptomic and phenotypic responses of the ear and the flag leaf in wheat has been reported by few authors under ambient CO$_2$ (Vicente et al., 2018) and high CO$_2$ conditions (Aranjuelo et al., 2008, 2011). Despite the response of leaf photosynthesis to elevated CO$_2$ having been detailed broadly, there has been little attention given to the photosynthetic performance of ears and other organs, such as glumes, under high (CO$_2$) in wheat. Therefore, the objective of this study was to analyze the impact of elevated atmospheric CO$_2$ on the photosynthetic machinery of wheat flag leaves and ears. For this purpose, we have characterized the concentration of target metabolites transcript levels of C and N metabolism genes, as well as other physiological parameters in both organs at grain filling stage.

MATERIALS AND METHODS

Plant Material and Experimental Design

The experiment was conducted with durum wheat plants (Triticum turgidum subsp. durum), using the genotype Amilcar, which is a commercial wheat cultivar widely used in the Navarra region. Wheat seedlings were vernalized for 1 month (during January 2017) at 4°C and then transplanted into 13 L pots.
with six plants per pot, containing a mixture of 2:2:1 of vermiculite:perlite:peat. The plants were grown at the University of Navarra campus (42°80′N, 1°66′W; Pamplona, Spain), at average ambient temperature of 20°C and 50% relative humidity. The study was conducted in four separated greenhouses with doors along the longitudinal walls to allow the access to plants inside, two greenhouses kept at ambient air CO₂ concentration [a(CO₂)] and the other two at elevated CO₂ [e(CO₂)]. In a(CO₂) conditions no CO₂ was added and CO₂ was close to 400 ± 20 µmol mol⁻¹. In the e(CO₂) treatment, the (CO₂) was close to 700 ± 20 µmol mol⁻¹ (24 h per day) by injecting pure CO₂ from the start of the experiment. The (CO₂) was continuously monitored using a Guardian Plus gas monitor (Edinburgh Instruments Ltd., Livingston, United Kingdom) whose signal controls the valve that injects the CO₂. The CO₂ used to increase CO₂ level was provided by Air Liquide (Valladolid, Spain). The isotopic composition (δ¹³C) within the elevated CO₂ level modules was −20.6‰ whereas in case of ambient CO₂ greenhouses δ¹³C values were −10.3‰. The pots were located separately in the middle of each greenhouse and they were rotated weekly to avoid edge effects. There were eight replicates (pots) in each treatment distributed in two greenhouses with the same (CO₂) concentration. The plants were surrounded by additional pots with in order to avoid edge effect, with an average distance of 5 cm between pots. Plants were watered with a complete Hoagland nutrient solution twice a week and with water once a week until harvesting.

Measurements of gas exchange and sampling to determination of carbon – C¹₂ isotopic composition, metabolic analysis and gene expression were conducted when plants reached 2 weeks after anthesis (Zadoc stage 65). This period frequently is more sensitive to environmental changes because coincides with the largest photosynthesizable contribution to grain filling (Aranjuelo et al., 2011). Determinations and sampling were carried out 2–5 h after sunrise. The flag leaves and ears collected were plunged immediately into liquid nitrogen and stored at −80°C for further analyses. The glumes were separated from the ears under liquid nitrogen.

Agronomic Determinations

When the plants reached the maturity stage, plant shoot, ears, and grains were collected. For this purpose, eight plants were harvested for each treatment combination. Collected samples were weighed after drying in an oven at 60°C during 48 h. Total dry matter (DM) was determined and included ear DM and shoot DM. Further, the agronomical parameters, thousand kernel weight (TKW), harvest index (HI = ratio between seed weight and total DM) and grain number were determined.

Gas Exchange Determinations

Gas exchange measurements were conducted 2 weeks after anthesis in the central segment of the flag leaves and complete ears of plants grown under both (CO₂) treatments. The net (An) and apparent gross photosynthesis – A(gross), stomatal conductance (gs), sub-stomatal CO₂ mole fraction (Ci) and electron transport rate (ETR) were determined at 25°C for each growth condition using a Li-Cor 6400 XP portable gas exchange photosynthesis system (LI-COR, 6400 XT, United States), with a photosynthetic photon flux density (PPFD) of 1200 µmol m⁻² s⁻¹. Photosynthesis was recorded at 400 and 700 µmol mol⁻¹ in each treatment. To taking into account the respiration rates, the sum between An plus dark respiration (Rd) (An + Rd) was defined as A(gross). To conduct dark respiration measurements the plants were dark-adapted for 4 h. Maximal carboxylation velocity by Rubisco (Vcmax) was determined using the method of Harley et al. (1992). To measure ear gas exchange, entire ears were placed in a homemade aluminum chamber (20 × 12 × 6 × 10⁻⁶ m³) connected to the LiCor 6400 XT gas exchange analyzer (Aranjuelo et al., 2011). Ear surface was determined in 2D area basis through a scanner. Within the chamber, ingoing air was passed through the chamber at a rate of 1 L min⁻¹. The molar fractions of CO₂ and humidity were measured with the infrared gas analyzer of the LiCor-6400XT. The CO₂ partial pressure was maintained constant with the infrared gas analyzer-controlled CO₂ injection system. To ensure steady-state conditions inside the chamber, the system was left to stabilize for a few minutes. An external light source composed of LED lights was placed around the chamber during the measurements, achieving a saturating PPFD of approximately 1200 µmol m⁻² s⁻¹ inside the chamber.

Carbon Isotope Composition Analysis

The stable C isotope composition (δ¹³C) was conducted 2 weeks after anthesis in leaves and glumes separated manually from the ear. For δ¹³C analysis of DM, approximately 1 mg of each sample was weighed into tin capsules and measured with an elemental analyzer coupled to an Isotope Ratio Mass Spectrometer (Delta C IRMS, ThermoFinnigan, Bremen, Germany) operating in continuous flow mode in order to determine the stable C¹₂/C¹³ isotope ratios of the same samples. The C¹³/C¹₂ ratios of plant material were expressed in δ notation (Coplen, 2008):

\[
\delta^{13}C = \left( \frac{^{13}C/^{12}C}_{\text{sample}} \right) / \left( ^{13}C/^{12}C \right)_{\text{standard}} - 1,
\]

where “sample” refers to plant material and “standard” to international secondary standards of known ¹³C/¹²C ratios (IAEA CH7 polyethylene foil, IAEA CH6 sucrose and USGS 40 L-glutamic acid) calibrated against Vienna Pee Dee Belemnite calcium carbonate (VPDB) with an analytical precision (SD) of 0.10‰.

Sugar and Organic Acid Contents

For sugar extraction, dried flag leaves and glumes were ground to a fine powder. About 25 mg of the powder were suspended and mixed in 1 ml of ethanol (80%) in an Eppendorf tube, then the samples were shaken in a thermomixer (90 min, 70°C, 1100 rpm) and centrifuged at 20,800 × g for 10 min at 22°C. The pellet was used to starch quantification and the supernatant was used for soluble sugar quantification. To solubilization of starch, the pellet was re-suspended in KOH 0.2N and starch extraction was done using an amyloglucosidase test kit (R-Biopharm AG, Darmstadt, Germany). Finally, the quantification was performed through absorbance measurements at 340 nm. Glucose, fructose and
glucose were determined in the supernatant obtained from flag leaves, glumes and grain organs using an ionic chromatographer (ICS-3000, Thermo Scientific™, United States).

The organic acids (OAs) malate, citrate, oxalate, and succinate were determined using isocratic ion chromatography in the supernatant fraction after ethanol (80% v/v) extraction for 25 min at 30°C in an ultrasonic bath. Subsequently, the extracts were filtered with Millex filters (Millipore, Billerica, MA, United States) and injected into a Dionex-DX500 (Dionex Corporation, CA, United States) system equipped with an ED40 electrochemical detector. An IonPac AS11 column connected to an ATC-1 protecting column and an AG11 pre-column (Dionex, Salt Lake City, UT, United States) was used.

**Nitrogen Concentration, Total Amino Acid and Soluble Protein Contents**

N concentration and total amino acid content (TAA) were determined in flag leaves, glumes, and grains from plants grown under $a$(CO$_2$) and $e$(CO$_2$). N elemental content was determined in dry and ground material at 60°C for 48 h based on sample dynamic combustion, using an elemental analyzer (EA1108, Carbo Erba Instrumentazione, Veneto, Italy). Total amino acids were determined in lyophilized, ground and weighed ($\approx$20 mg) samples. The ground material was suspended in 500 µl of ethanol (80% v/v), shaken in thermomixer (60 min, 80°C, 600 rpm) and centrifuged (10 min, 4°C, 14,000 × g). After centrifugation, the supernatant was collected and the ethanol was evaporated at Speed vacuum for 3 h. The residue obtained is re-suspended in 100 µl of deionized water and centrifuged. The supernatant was used to derivatization with an ACCQ-Fluor™ Reagent kit (WATER, United States) based on borate buffer, acetonitrile and AQC derivatizing reagent (6-aminoquinolyl-N-hydroxysuccinimidyl carbamate) using High Performance Liquid Chromatography (HPLC). The total soluble protein (TSP) concentration was also measured in these samples by the Bradford method (Bradford, 1976).

**Quantitative Real-Time PCR Analysis**

RNA from 100 mg of frozen plant material (flag leaves and glumes) was isolated as described by Morcuende et al. (1998). A 10 µg quantity of RNA per sample was treated with Turbo DNase (Ambion) according to the manufacturer’s instructions, and subsequently the absence of genomic DNA was tested by PCR using a primer pair for the PolA1 gene (AB647308) as described in Vicente et al. (2015). Afterward, RNA integrity was checked on a 1.5% (v/v) agarose gel prior to and after DNase digestion. cDNA was synthesized using SuperScript III reverse transcriptase (Invitrogen GmbH) according to the manufacturer’s instructions. Only cDNA preparations that yielded similar Ct values for the reference gene were used for comparing transcript levels of target genes. Polymerase chain reactions were performed in a 384-well optical plate using a sequence detection system (ABI PRISM 7900 HT, Applied Biosystems), with SYBR Green PCR Master Mix (Applied Biosystems), diluted cDNA (1:40) and the specific primers of each gene (Vicente et al., 2015). The thermal profile was as follows: 50°C for 2 min, 95°C for 10 min, 40 cycles of 95°C for 15 s and 60°C for 1 min, and a final step of 95°C for 15 s and 60°C for 15 s to obtain the dissociation curve. Three biological replicates were used for quantification analysis and two technical replicates were analyzed for each biological replicate.

The gene encoding ADP-ribosylation factor (Unigene Ta2291) was used as a reference gene for normalizing gene expression results after validating its expression stability in our growth conditions, in agreement with Vicente et al. (2015). We evaluated the transcript levels for 30 genes associated with photosynthesis, carbohydrate metabolism, glycolysis, the tricarboxylic acid (TCA) cycle and N assimilation in flag leaves, and glumes using the primers described in Vicente et al. (2015). The specificity of PCR amplification was confirmed by the presence of unique amplicons of the expected length on 3.5% (w/v) agarose gels. All genes analyzed and their abbreviations are shown in Supplementary Table 1. The values of the cycle threshold (Ct) were calculated using SDS 2.4 software (Applied Biosystems). Relative gene expression was quantified using the comparative Ct method $2^{-\Delta\Delta C_{t}}$ (Schmittgen and Livak, 2008), and the data were presented as the log$_{2}$ ratio of e(CO$_2$) over a(CO$_2$) for each organ.

**Statistical Analysis**

Statistical analyses for the effects of e(CO$_2$) relative to a(CO$_2$) and/or the differences between organs (flag leaves and ears or glumes) were performed through one- and two-factor analyses of variance (ANOVA) in R environment (R® v.3.4.2, 2017; Boston, MA–Seattle, WA, United States). Differences between a(CO$_2$) and e(CO$_2$) treatments means and/or organs were assessed using Tukey’s HSD test. Significance were accepted at $p < 0.05$. All figures were generated in Sigma-Plot 11.0 program (Systat Software Inc.).

**RESULTS**

**Growth and Physiological Parameters**

Plant growth data (Table 1) showed that exposure to e(CO$_2$) increased plant growth. Such increase was linked to an stimulation in grain yield (explained by an enhancement in grain number) of e(CO$_2$).

Moreover, the magnitude of the CO$_2$ gradient (Ca-Ci, Ca:


**TABLE 1** | Effect of a(CO\textsubscript{2}) and e(CO\textsubscript{2}) (400 vs. 700 µmol mol\textsuperscript{-1}) on shoot, ear, total and grain biomass, thousand kernel weight (TKW), harvest index (HI), and grain number of durum wheat plants.

| CO\textsubscript{2} | Shoot DM | Ear DM | Total DM | Grain DM | TKW | HI | Grain number |
|---------------------|----------|--------|----------|----------|-----|----|-------------|
| a(CO\textsubscript{2}) | 12.70 ± 0.67 | 10.50 ± 0.82 | 24.72 ± 1.72 | 6.38 ± 0.38 | 47.69 ± 3.44 | 0.26 ± 0.00 | 141.92 ± 23.40 |
| e(CO\textsubscript{2}) | 16.63 ± 0.90 | 17.97 ± 1.23 | 34.60 ± 1.66 | 11.81 ± 1.09 | 40.09 ± 2.43 | 0.34 ± 0.02 | 298.60 ± 30.15 |

*p*-Value 0.006 0.001 0.002 0.001 0.101 0.006 0.002

Each value represents the mean ± SE (n = 3–4). The *p*-value in bold means significant differences between (CO\textsubscript{2}) in each organ.

Concentration of CO\textsubscript{2} at ambient) was stronger under e(CO\textsubscript{2}) (362.8 µmol mol\textsuperscript{-1}) than a(CO\textsubscript{2}) (245.7 µmol mol\textsuperscript{-1}) which would affect the isotope discrimination. Although no differences between CO\textsubscript{2} conditions were observed in \( V_{\text{Cmax}} \), at the organ level it was higher in ears than flag leaves (Figure 1D). The C is isotopic composition (\( \delta^{13}C \)) decreased under e(CO\textsubscript{2}) compared to a(CO\textsubscript{2}) for both flag leaves and glumes (46 and 60%, respectively). However, the leaves showed lower \( \delta^{13}C \) values (more negative) than glumes under a(CO\textsubscript{2}) (Figure 1F).

**Total Soluble Carbohydrates and Starch Content**

The total soluble carbohydrates (TSCs), which comprise the sum of glucose, fructose, and sucrose concentrations, tended to be higher in plants grown under e(CO\textsubscript{2}) (Table 2) irrespective of the organ evaluated. In comparison to the glumes and grains, the flag leaves showed a greater increase in TSCs [c.a. 136% at e(CO\textsubscript{2}) rather a(CO\textsubscript{2})]. Among the individual sugars, glucose was not significantly different between the CO\textsubscript{2} conditions, but...
TABLE 2 | Effect of $a$(CO$_2$) and $e$(CO$_2$) (400 vs. 700 µmol mol$^{-1}$) on glucose, fructose, sucrose, total soluble carbohydrates (TSCs) and starch contents in flag leaf, glume, and grain of durum wheat plants.

| Organ     | $CO_2$            | Glucose (mg g$^{-1}$ DM) | Fructose (mg g$^{-1}$ DM) | Sucrose (mg g$^{-1}$ DM) | TSC (mg g$^{-1}$ DM) | Starch (mg g$^{-1}$ DM) |
|-----------|-------------------|--------------------------|---------------------------|--------------------------|----------------------|--------------------------|
| Flag leaf | Ambient $CO_2$    | 11.82 ± 0.51             | 8.37 ± 0.66               | 71.39 ± 4.97             | 91.58 ± 4.52         | 4.96 ± 0.09              |
|           | Elevated $CO_2$  | 13.20 ± 5.93             | 27.21 ± 6.54              | 175.70 ± 10.58           | 216.11 ± 14.58       | 4.37 ± 0.22              |
|           | $p$               | 0.828                    | 0.046                     | <0.001                   | 0.001                | 0.066                    |
| Glume     | Ambient $CO_2$    | 1.54 ± 0.32              | 3.13 ± 0.18               | 4.21 ± 0.66              | 8.88 ± 0.76          | 1.47 ± 0.08              |
|           | Elevated $CO_2$  | 3.47 ± 0.84              | 1.60 ± 0.43               | 7.47 ± 2.03              | 12.54 ± 3.16         | 2.77 ± 0.11              |
|           | $p$               | 0.098                    | 0.031                     | 0.202                    | 0.323                | -0.001                   |
| Grain     | Ambient $CO_2$    | 100.67 ± 4.18            | 46.17 ± 2.84              | 110.51 ± 8.30            | 257.34 ± 13.91       | 2.67 ± 0.04              |
|           | Elevated $CO_2$  | 117.61 ± 9.33            | 127.39 ± 16.87            | 40.53 ± 0.95             | 285.54 ± 20.64       | 2.81 ± 0.02              |
|           | $p$               | 0.173                    | 0.009                     | 0.001                    | 0.321                | 0.041                    |

Each value represents the mean ± SE ($n = 3–4$). The $p$-value in bold means significant differences between (CO$_2$) in each organ. Levels of significance: ns, not significant; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

fructose increased under e(CO$_2$) in leaves (225%) and grains (176%) and decreased in glumes (49%) relative to $a$(CO$_2$). The sucrose content at e(CO$_2$) increased significantly in leaves (146%), decreased in grains (63%) but was not significantly different in glumes. The starch content increased in glumes and grains under elevated CO$_2$ (88 and 5%, respectively).

**Organic Acid Contents**

In order to further compare the response of C and/or energy metabolism under e(CO$_2$), four C-rich OAs related to the TCA cycle and other metabolic pathways were analyzed in flag leaves, glumes, and grains, namely succinate, malate, $\alpha$-ketoglutarate, and citrate (Figure 2). Their contents, designated here as total OA content, tended to decrease under e(CO$_2$) in flag leaves and glumes. Significantly, succinate, malate, and citrate in leaves and malate in glumes were lower under e(CO$_2$), although the malate and citrate contents were higher in grains (Figure 2B). At the organ level, for all OAs measured, grains had the highest values relative to leaves and glumes, while, glumes had the lowest content compared to the other organs, except for $\alpha$-ketoglutarate (Figure 2A).

**Nitrogen Concentration, Total Amino Acid and Total Soluble Protein Contents**

In general, N concentration and TAA content were lower under e(CO$_2$) than $a$(CO$_2$), while TSP content was higher (Figure 3). In comparison to leaves and glumes, N and TAA contents were higher in grains than leaves and glumes, while TSP content was higher in leaves than glumes. Although N and TAA contents tended to decrease and TSP to increase under e(CO$_2$) in leaves,
the differences were not statistically significant. Nevertheless, the effect of \( e(\text{CO}_2) \) was remarkable in glumes; in this organ, the N decreased 33% and TSP increased 43% under \( e(\text{CO}_2) \) compared to \( a(\text{CO}_2) \). In grains, N decreased 19% under \( e(\text{CO}_2) \) and no effects were found in the TAA content at different \( \text{CO}_2 \) concentrations.

**Relative Gene Expression in Flag Leaves and Glumes**

In this study, the relative expression of genes linked to photosynthesis, carbohydrate metabolism, glycolysis, the TCA cycle, and N metabolism were analyzed to study the effect of \( e(\text{CO}_2) \) over \( a(\text{CO}_2) \) for each organ. In general, most of the genes analyzed were substantially down-regulated under \( e(\text{CO}_2) \) in flag leaves and glumes. Two genes linked to carbohydrate metabolism (sucrose:sucrose 1-fructosyltransferase, 1SST) and glycolysis (pyrophosphate-fructose-6-phosphate 1-phosphotransferase, PFP) were significantly down-regulated under \( e(\text{CO}_2) \) in flag leaves, while other genes related to light harvesting and the Calvin–Benson cycle tended to decrease. Cytosolic glutamine synthetase (GS1) gene expression was significantly up-regulated in flag leaves under \( e(\text{CO}_2) \), and the expression of other genes related to respiration, starch synthesis and carbonic anhydrases tended to increase (Figure 4). On the other hand, there was greater repression of transcript levels in the glumes than the flag leaves under \( e(\text{CO}_2) \), indicating a
FIGURE 4 | Relative transcript levels of durum wheat plants exposed to e(CO$_2$) in flag leaf and glume organs. Asterisks indicate a significant difference between (CO$_2$) treatments: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

general repression of glume metabolism. While the plastidial carbonic anhydrase (CA2) was significantly up-regulated in e(CO$_2$), the genes related to sugar degradation and respiration (fructan 1-exohydrolase, 1FEH; hexokinase, HXK; pyruvate kinase, PK; E1 component α-subunit of mitochondrial pyruvate dehydrogenase complex, PDC; mitochondrial NAD-dependent isocitrate dehydrogenase, IDH; and E1 subunit of 2-oxoglutarate dehydrogenase complex, OGDC), and N metabolism (cytosolic aspartate aminotransferase, cAAT) were significantly down-regulated.

DISCUSSION

**Elevated CO$_2$ Alters Gas Exchange, Carbon and Nitrogen Metabolism**

It is well documented that in C$_3$ plants e(CO$_2$) increases leaf photosynthesis and reduce stomatal conductance (Ainsworth and Rogers, 2007), which in absence of soil water or nitrogen limitations, increases plant biomass, and yield (Reich et al., 2014; Gray et al., 2016). However, little is known about other photosynthetic organs, as ears. The current study showed that, in agreement with above mentioned studies, the net (An) and apparent gross photosynthesis – A(gross) exhibited higher values at e(CO$_2$) in both flag leaves and ear organs compared to a(CO$_2$), which contributed to increase grain yield and plant biomass of plants under e(CO$_2$). The stimulation of An and plant growth under e(CO$_2$) conditions has been documented by several authors (Zhu et al., 2008; Aranjuelo et al., 2015; Dusenge et al., 2019). In additional physiologic analyses, did not detect differences in gs and ETR at e(CO$_2$) in flag leaves, but Ci increased under e(CO$_2$) with a strong gradient of CO$_2$ (Figure 1). These results indicate that the enhancement of An by e(CO$_2$) may be caused predominantly by the increase in (CO$_2$) at the site of CO$_2$ fixation, which increases the CO$_2$/O$_2$ ratio, thus promoting the efficiency of Rubisco carboxylation in leaf tissues (Gamage et al., 2018). Further, it should be also considered that due to the fact that under e(CO$_2$) air is enriched in $^{12}$CO$_2$, this would contribute to increase the absolute concentration of $^{12}$CO$_2$ at the active site. In spite of changes in $\delta^{13}$C due to the CO$_2$ treatment, there were
differences associated with each organ. The discrimination of $^{13}$C depends on the ratio of the assimilation rate to CO$_2$-diffusive conductance (Sanchez-Bragado et al., 2014a). In our study, the glumes showed higher values of $^{13}$C than the flag leaves, at least under $a$(CO$_2$). This could be due to lower permeability to gas diffusion in the ears compared to leaves, which caused the increase in $^{13}$C in this organ (Sanchez-Bragado et al., 2014a). However, no differences in $^{13}$C were observed in the flag leaves and glumes at $e$(CO$_2$), which there is enough $^{13}$C supply.

Additionally, plant respiration rates ($Rd$) may be altered under $e$(CO$_2$) conditions (Gamage et al., 2018). To distinguish the differential effect of $e$(CO$_2$) in respiration of flag leaf and ear, the $A$(gross) was measured. In this study, the gross photosynthesis increased in the ears (Figure 1A) due to higher respiration rates compared to the flag leaves at $e$(CO$_2$). Previous studies have reported greater $Rd$ values than $A$n in the ear, as a consequence of the respiration of developing grains and non-photosynthetic tissues (Araus et al., 1993). The fact that under elevated (CO$_2$) grain DM and carbohydrate content increased, would help to explain the larger respiration rates of those plants. Studies in ears of cereals with C$_3$ photosynthesis has suggested that the high levels of CO$_2$ released by the respiration of the developing grains can be re-fixed in the ear itself (Tambussi et al., 2007; Sanchez-Bragado et al., 2020). This re-fixation capacity might confer an important role on the ears of providing assimilates for grain filling (Araus et al., 1993; Bort et al., 1996; Tambussi et al., 2007; Sanchez-Bragado et al., 2014a; Zhou et al., 2014; Vicente et al., 2016). Zhu et al. (2009) suggested that the role of the ears to supply assimilates during grain-filling could be larger under $e$(CO$_2$) conditions due to the acceleration of senescence in flag leaves compared to the ears.

As consequence of the increase in $A$n at $e$(CO$_2$) an accumulation of sugars takes place (Zhu et al., 2009; Watanabe et al., 2014; Aranjuelo et al., 2015; Vicente et al., 2015; Dusenge et al., 2019), as observed in the current study for fructose, sucrose and TSCs (Table 2). However, sugar accumulation was more substantial in flag leaves than glumes, which may be due to reduced or insufficient sink capacity in the ears and grains under these conditions (Leakey et al., 2009). The fact that glumes are “closer” to grains (target sink) could also affect the carbohydrate balance of the ear. Within this context, it should be also considered that, the changes in metabolic compounds, as sugars, are dynamics respect the time of the day and can be modified constantly according the environmental conditions. In this sense, $e$(CO$_2$) can alter the C source-sink balance in the plants (Fabre et al., 2019). For this reason, in the present study we consider a transitory change and/or accumulation of metabolites, such as the sugars, and the results represent only a unique time of the day. Having this assumption in mind, while the starch content tended to decrease in leaves, it increased in glumes and grains, possibly as a strategy to release soluble sugars and translocate them away from leaves, with a consequent reduction in starch accumulation (Gamage et al., 2018). Furthermore, as mentioned above, high concentrations of sugars can be related to the stimulation of respiration, as observed in Figure 1A, with the sugars acting as substrates for respiration (Dusenge et al., 2019), especially in the ears. Nevertheless, carbohydrate accumulation is not the only factor that alters respiration rate, various intermediates of the respiration system, such as OA, also have a similar influence. Thus, changes in OA availability might affect respiration rates under $e$(CO$_2$). In our study, the OA content tended to decrease at $e$(CO$_2$) (Figure 2), which contrasts with increases that have been reported for the day period (Watanabe et al., 2014). Interestingly, Sweetlove et al. (2010) indicated that OA may be consumed as a respiratory substrate even in the light, highlighting that the TCA cycle is not only important at night. Moreover, increases in citrate levels, especially in grains, can serve as C skeletons for the synthesis of N-rich compounds, such as amino acids and proteins (Watanabe et al., 2014).

In this study, the N and TAA content decreased in flag leaves, glumes and grains of plants grown under $e$(CO$_2$), in agreement with earlier reports in durum (Aranjuelo et al., 2015; Vicente et al., 2015) and spring wheat (Lopes et al., 2006; Del Pozo et al., 2007; Andrews et al., 2019). Such a decline in N concentration can be explained by a dilution of N in tissues due to increase in C assimilation (Vicente et al., 2015), reduction in plant N demand (Stitt and Krapp, 1999), a decrease in N assimilation (Bloom, 2015) related to the inhibition of leaf primary assimilation (Vicente et al., 2016) and/or photorespiration (Bloom et al., 2012), and acceleration of leaf N remobilization (Aranjuelo et al., 2015). Our results showed an increase in TPS content under $e$(CO$_2$), which can be associated with a reduction in amino acid content due to its utilization in protein synthesis (Lopes et al., 2006). Moreover, the differences between CO$_2$ treatments for N and TSP content were significant in glumes, but not in flag leaves (Figure 3). This could have been due to the flag leaves undergoing an accelerated senescence under $e$(CO$_2$), which causes a greater N translocation from leaf to grain to support the N demand during grain filling (Zhu et al., 2009), with considerable depletion in N content in glumes. Our results support earlier observations that the glumes have a prominent role in N feeding of the grain (Lopes et al., 2006).

**Photosynthesis Acclimation Confirmed by Gene Expression**

The initial stimulation of photosynthetic rate in C$_3$ plants under $e$(CO$_2$) is well characterized and determined in the current study. However, it has been previously observed that photosynthesis decreases in response to prolonged exposure to $e$(CO$_2$) (Martínez-Carrasco et al., 2005; Del Pozo et al., 2007; Aranjuelo et al., 2011, 2015; Vicente et al., 2015). This phenomenon is known as “photosynthetic acclimation” and it is characterized by the suppression of several genes expression (Gamage et al., 2018). The present study analyzed the relative gene expression of photosynthesis, carbohydrate metabolism, glycolysis, TCA cycle and N metabolism in both flag leaf and glume organs. In agreement with previous manuscript, our study showed that the expression level of most of genes related to photosynthesis [such as Rubisco, Rubisco activase, and Ferredoxin-NADP(H) oxidoreductase] tended to be down-regulated in leaf and glume tissues under $e$(CO$_2$) (Figure 4). Previous studies have reported the down-regulation of genes
encoding for both small and large sub-unit (rbcS and rbcL) at e(CO2) (Bokhari et al., 2007; Aranjuelo et al., 2011; Takatani et al., 2014; Vicente et al., 2015) and this declination is often associated to the increase in carbohydrate content and lower plant N concentrations (Vicente et al., 2016, 2015). Furthermore, in the current study the relative gene expression of carbonic anhydrases (CA1 and CA2) was up-regulated under e(CO2) relative to a(CO2), and mainly in the grumes (Figure 4). These enzymes catalyze the reversible conversion of CO2 to HCO3− and have been related to the regulation of mesophyll conductance (Evans et al., 2009). Our results resembled previous findings (Aranjuelo et al., 2011), suggesting that the up-regulation in expression of those genes under e(CO2) might ensure the supply of CO2 to the chloroplasts, due to the increase in Ci and gradient Ca-Ci under such conditions.

The e(CO2) induced the carbohydrate accumulation in leaves and grumes. We observed a down-regulation of the expression of genes related to carbohydrate metabolism in plants grown in e(CO2), while the sugars increased. The accumulation under such conditions can be associated to the repression of genes related to carbohydrate degradation, such as Fructan 1-exohydrolase (FEH), as reported in durum wheat (Vicente et al., 2015). A similar pattern of changes was also found in the expression of the FEH gene in grumes. The repression of gene expression of glycolysis and TCA enzymes, mainly in grumes, was observed in this study despite the increase in Rd, it can be due to the carbohydrate accumulation at e(CO2) (Markelz et al., 2014). These results are consistent with Vicente et al. (2015) and Markelz et al. (2014). Another relevant issue within this context is the close interaction among TCA, C, and N metabolism. Several TCA intermediaries (such as malate, α-oxoglutarate) are required to regulate the N assimilation and amino acid biosynthesis, mainly asparagine, aspartic acid, glutamine, and glutamic acid, in responses of changes of C metabolism (Morcuende et al., 1998). In general, these intermediaries provide C skeletons for N assimilation (Watanabe et al., 2014). Taking this into account, the repression of N metabolism genes observed in grumes at e(CO2) could be associated to repression of TCA genes as precursors of amino acids synthesis. Finally, the fact that, in e(CO2) grumes, total protein was increased could be also reflecting fast assimilation of amino acids in proteins in those plants. The slight increase in GS1 would contribute to explain such response. In case of flag leaves, the strong up-regulation on GS1 would remark that was up-regulated (Lopes et al., 2006) under e(CO2) in flag leaves, which could indicate an assimilation of N in proteins. The decrease in the expression of most of the genes related to primary assimilation in grumes, together with the low N levels and amino acid content at e(CO2) suggested a fast remobilization during grain filling, as reported in durum wheat by Aranjuelo et al. (2015).

CONCLUSION

Our results indicate a differential response of ears relative to flag leaves in durum wheat plants grown under e(CO2). The higher ear gross photosynthesis, together with leaf photosynthesis enhancement, explained the increase in plant biomass and yield under e(CO2). The current study also showed that, in general, ears showed higher apparent gross photosynthesis and respiration rates that contributed to increase plant biomass. However, e(CO2) plants also showed lower accumulation of soluble sugars, apparently faster N remobilization and assimilation, and stronger repression of genes compared to the flag leaf. Our findings also support the concept that photosynthetic acclimation to e(CO2) was driven by accumulation of carbohydrates, a reduction in the N concentration and repression of genes related to photosynthesis, glycolysis, the TCA cycle and the N assimilation pathway, with the changes in gene expression being more pronounced in grumes than leaves. This highlights the importance of investigating the close interaction between C and N metabolic pathways at the biochemical and transcriptional level in the different organs that contribute to grain development, and under the conditions anticipated for future changes in climate. Further, the relevance of N availability on adjustment of C metabolism (and crop yield) is a major issue that should be considered. This study provides much needed information on how e(CO2) impacts grain filling and will help to formulate strategies to optimize wheat grain yield and nutritional quality.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

AG: writing, performing lab, and data analysis. RV and IJ: lab analyses, writing, and review. RS-B: lab analyses and review. RM: supervision, writing, and review. NG: supervision and review. IA: supervision, editing and review. All authors read and approved the present manuscript.

FUNDING

This research was supported by the Spanish Innovation and Universities Ministry (PCIN-2017-007) and the Ministry of Education and Sciences (grants PID2019-107154RB-100; PID2019-110445RB-100). RV was the recipient of an FPI fellowship from the Spanish Ministry of Economy and Competitiveness (BES-2010-031029). RS-B was supported by the Juan de la Cierva program JDC-Formación (FJCI-2016-28164).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2020.587958/full#supplementary-material
REFERENCES

Abebe, T., Melaika, K., Berg, V., and Wise, R. P. (2010). Drought response in the spikes of barley: gene expression in the lemma, palea, awn, and seed. Funct. Integr. Genomics 10, 191–205. doi: 10.1007/s10142-009-0149-4

Ainsworth, E. A., and Rogers, A. (2007). The response of photosynthesis and stomatal conductance to rising [CO2]: mechanisms and environmental interactions. Plant Cell Environ. 30, 258–270. doi: 10.1111/j.1365-3040.2007.01641.x

André, M., and Ducloûx, H. (1993). Interaction of CO2 enrichment and water limitations on photosynthesis and water efficiency in wheat. Plant Physiol. 91, 103–112.

Andrews, M., Condron, L. M., Kemp, P. D., Topping, J. F., Lindsey, K., Hodge, S., et al. (2019). Elevated CO2 effects on nitrogen assimilation and growth of C3 vascular plants are similar regardless of N-form assimilated. J. Exp. Bot. 70, 683–690. doi: 10.1093/jxb/ery371

Aranjuelo, I., Cabrera, L., Morcuende, R., Avice, J.-C., Nogués, S., Araus, J. L., et al. (2011). Does ear C sink strength contribute to overcoming photosynthetic acclimation of wheat plants exposed to elevated CO2? J. Exp. Bot. 62, 3957–3969. doi: 10.1093/jxb/ert095

Aranjuelo, I., Erice, G., Sanz-Sáez, A., Abadie, C., Gilard, F., Gil-quintana, E., et al. (2015). Differential CO2 effect on primary carbon metabolism of flag leaves in durum wheat (Triticum durum Desf.). Plant Cell Environ. 38, 2780–2794. doi: 10.1111/pce.12513

Aranjuelo, I., Sánchez-Díaz, M., and Nogués, S. (2008). Carbon partitioning in N2 fixing Medicago sativa plants exposed to different CO2 and temperature conditions. Funct. Plant Biol. 35, 306–317. doi: 10.1071/fp07296

Araus, J. L., Brown, H. R., Febrero, A., Bort, J., and Serret, M. D. (1993). Ear photosynthesis, carbon isotope discrimination and the contribution of respiratory CO2 to differences in grain mass in durum wheat. Plant Cell Environ. 16, 383–392. doi: 10.1111/j.1365-3040.1993.tb00884.x

Bloom, A. J. (2015). Photoreception and nitrate assimilation: a major intersection between plant carbon and nitrogen. Photophys. Res. 123, 117–127. doi: 10.1007/s11120-014-0056-y

Bloom, A. J., Rubio, J. S., Randall, L., Rachmilevitch, S., Cousins, A. B., and Carlisle, E. A. (2012). CO2 enrichment inhibits shoot nitrate assimilation in C3 but not C4 plants and slows growth under nitrate in C3 plants. Ecology 93, 355–367. doi: 10.1890/11-0485.1

Blum, A., Mayer, J., and Golan, G. (1988). The effect of grain number per ear (sink size) on source activity and its water-relationships in wheat. J. Exp. Bot. 39, 106–114. doi: 10.1093/jxb/39.1.106

Bokhari, S. A., Wan, X.-Y., Yang, Y.-W., Zhou, L., Tang, W.-L., and Liu, J.-Y. (2007). Protonic response of rice seedling leaves to elevated CO2 levels. J. Proteome Res. 6, 4624–4633. doi: 10.1021/pr070524t

Borrill, P., Fahy, B., Smith, A. M., and Uauy, C. (2015). Wheat grain filling is limited by assimilation and stomatal conductance. Plant Physiol. 168, 451–457. doi: 10.1104/pp.114.233054.1986.tb03381.x

Leakey, A. D. B., Ainsworth, E. A., Bernacchi, C. J., Rogers, A., Long, S. P., and Ort, D. R. O. (2009). Elevated CO2 effects on plant carbon, nitrogen, and water relations: six important lessons from FACE. J. Exp. Bot. 60, 2859–2876. doi: 10.1093/jxb/erp096

Li, X., Ullat, A., Shokat, S., Liu, S., Zhu, X., and Liu, F. (2019). Responses of carbohydrate metabolism enzymes in leaf and spike to CO2 elevation and nitrogen fertilization and their relations to grain yield in wheat. Environ. Exp. Bot. 164, 149–156. doi: 10.1016/j.envexpbot.2019.05.008

Long, S. P., Ainsworth, E. A., Rogers, A., and Ort, D. R. (2004). Rising atmospheric carbon dioxide: plants FACE the Future. Annu. Rev. Plant Biol. 55, 591–628. doi: 10.1146/annurev.plant.55.031903.141610

Lopes, M. S., Cortadelas, N., Kichey, T., Dubois, F., Habash, D. Z., and Araus, J. L. (2006). Wheat nitrogen metabolism during grain filling: comparative role of glumes and the flag leaf. Planta 225, 165–181. doi: 10.1007/s00425-006-0338-5

Markelz, R. J. C., Vosseller, L. N., and Leakey, A. D. B. (2014). Developmental stage specificity of transcriptional, biochemical and CO2 efflux responses of leaf dark respiration to growth of Arabidopsis thaliana at elevated [CO2]. Plant Cell Environ. 37, 2542–2552. doi: 10.1111/pce.12332

Martínez-Carrasco, R., Pérez, P., and Morcuende, R. (2005). Interactive effects of elevated CO2 and temperature on photosynthesis of wheat grown under temperature gradient tunnels. Environ. Exp. Bot. 54, 49–59. doi: 10.1016/j.envexpbot.2004.05.004

Maydup, M. L., Antonietta, M., Guialet, J. J., Graciano, C., López, J. R., and Tambussi, E. A. (2010). Field Crops Research The contribution of ear photosynthesis to grain filling in bread wheat (Triticum aestivum L.). Field Crops Res. 119, 48–58. doi: 10.1016/j.fcr.2010.06.014

Molero, G., Joyron, R., Pinera-Chavez, F. J., Gardiner, L.-J., Rivera-Amado, C., Hall, A., et al. (2019). Elucidating the genetic basis of biomass accumulation and radiation use efficiency in spring wheat and its role in yield potential. Plant Biotechnol. J. 17, 1276–1288. doi: 10.1111/pbi.13052
Morcuende, R., Krapp, A., Hurry, V., and Stitt, M. (1998). Sucrose-feeding leads to increased rates of nitrate assimilation, increased rates of \( \alpha \)-oxoglutarate synthesis, and increased synthesis of a wide spectrum of amino acids in tobacco leaves. *Plant Cell* 206, 394–409. doi: 10.1007/s004250050415

Morgan, J. M. (1980). Osmotic adjustment in the spikelets and leaves of wheat. *J. Exp. Bot.* 31, 655–665. doi: 10.1093/jxb/31.2.655

Noua/Esrl (2020). *Trends in Atmospheric Carbon Dioxide*. Boulder, CO: ESRL.

Paul, M. J., and Foyer, C. H. (2001). Sink regulation of photosynthesis. *J. Exp. Bot.* 52, 1383–1400. doi: 10.1093/jexbot/52.360.1383

Reich, P. B., Hobbie, S. E., and Lee, T. D. (2014). Plant growth enhancement by elevated CO \(_2\) eliminated by joint water and nitrogen limitation. *Nat. Geosci.* 7, 920–924. doi: 10.1038/ngeo2284

Sanchez-Bragado, R., Elazab, A., Zhou, B., Serret, M. D., Bort, J., Nieto-Taladriz, M. T., et al. (2014a). Contribution of the ear and the flag leaf to grain filling in durum wheat inferred from the carbon isotope signature: genotypic and growing conditions effects. *J. Integr. Plant Biol.* 56, 444–454. doi: 10.1111/jipb.12106

Sanchez-Bragado, R., Molero, G., Reynolds, M. P., and Araus, J. L. (2014b). Relative contribution of shoot and ear photosynthesis to grain filling in wheat under good agronomical conditions assessed by differential organ 513C. *J. Exp. Bot.* 65, 5401–5413. doi: 10.1093/jxb/eru298

Sanchez-Bragado, R., Molero, G., Reynolds, M. P., and Araus, J. L. (2016). Photosynthetic contribution of the ear to grain filling in wheat: a comparison of different methodologies for evaluation. *J. Exp. Bot.* 67, 2787–2798. doi: 10.1093/jxb/erw116

Sanchez-Bragado, R., Vicente, R., Molero, G., Serret, M. D., Maydun, M. L., and Araus, L. (2020). New avenues for increasing yield and stability in C3 cereals: exploring ear photosynthesis. *Curr. Opin. Plant Biol.* 13, 1–12. doi: 10.1016/j.pbi.2020.01.001

Schmittgen, T. D., and Livak, K. J. (2008). Analyzing real-time PCR data by the comparative CT method. *Nat. Protoc.* 3, 1101–1108. doi: 10.1038/nprot.2008.73

Sourdille, P., Cadalen, T., Gay, G., Gill, B., and Bernard, M. (2002). Molecular and physical mapping of genes affecting awniness in wheat. *Plant Breed.* 121, 320–324. doi: 10.1046/j.1439-0523.2002.728336.x

Stitt, M., and Krapp, A. (1999). The interaction between elevated carbon dioxide and nitrogen nutrition: the physiological and molecular background. *Plant Cell Environ.* 22, 583–621. doi: 10.1046/j.1365-3040.1999.00386.x

Sweetlove, L. J., Beard, K. F. M., Nunes-Nesi, A., Fernie, A. R., and Ratcliffe, R. G. (2010). Not just a circle: flux modes in the plant TCA cycle. *Trends Plant Sci.* 15, 462–470. doi: 10.1016/j.tplants.2010.05.006

Takatani, N., Ito, T., Kiba, T., Mori, M., Miyamoto, T., Maeda, S.-I., et al. (2014). Effects of high CO \(_2\) on growth and metabolism of Arabidopsis seedlings during growth with a constantly limited supply of nitrogen. *Plant Cell Physiol.* 55, 281–292. doi: 10.1093/pcp/pct186

Tambussi, E. A., Bort, J., Guiazet, J. J., Noguès, S., and Araus, J. L. (2007). The photosynthetic role of ears in C3 cereals: metabolism, water use efficiency and contribution to grain yield. *Crit. Rev. Plant Sci.* 26, 1–16. doi: 10.1080/0735268060147901

Tambussi, E. A., Noguès, S., and Araus, J. L. (2005). Ear of durum wheat under water stress: water relations and photosynthetic metabolism. *Planta* 221, 446–458. doi: 10.1007/s00425-004-1455-7

Teramura, A. H., Sullivan, J. H., and Ziska, L. H. (1990). Interaction of elevated ultraviolet-B radiation and CO \(_2\) on productivity and photosynthetic characteristics in wheat, rice, and soybean. *Plant Physiol.* 94, 470–475. doi: 10.1104/pp.94.2.470

Vergara-Diaz, O., Vatter, T., Vicente, R., Obata, T., Nieto-Taladriz, M. T., Aparicio, N., et al. (2020). Metabolome profiling supports the key role of the spike in wheat yield performance. *Cells* 9, 2–25. doi: 10.3390/cells9041025

Vicente, R., Perez, P., Martinez-Carrasco, R., Reil, F., Renn, J. E., Watanabe, M., et al. (2016). Metabolic and transcriptional analysis of Durum wheat responses to elevated CO \(_2\) at low and high nitrate supply. *Plant Cell Physiol.* 57, 2133–2146. doi: 10.1093/pcp/pcc131

Vicente, R., Perez, P., Martinez-Carrasco, R., Usadel, B., Kostadinova, S., and Morcuende, R. (2015). Quantitative RT – PCR platform to measure transcript levels of C and N metabolism-related genes in Durum wheat: transcript profiles in elevated [CO \(_2\)] and high temperature at different levels of N supply. *Plant Cell Physiol.* 56, 1556–1573. doi: 10.1093/pcp/pct079

Vicente, R., Vergara-Diaz, O., Medina, S., Chai, F., Kefauver, S. C., Bort, J., et al. (2018). Durum wheat ears perform better than the flag leaves under water stress: gene expression and physiological evidence. *Environ. Exp. Bot.* 153, 271–285. doi: 10.1016/j.envexpbot.2018.06.004

Watanabe, C. K., Sato, S., Yanagisawa, S., Uesono, Y., Terashima, I., and Noguchi, K. (2014). Effects of elevated CO \(_2\) on levels of primary metabolites and transcripts of genes encoding respiratory enzymes and their diurnal patterns in Arabidopsis italiana: possible relationships with respiratory rates. *Plant Cell Physiol.* 55, 341–357. doi: 10.1093/pcp/pct185

Wechsung, F., Garcia, R. L., Wall, G. W., Kartschall, T., Kimball, B. A., Michaelis, P., et al. (2000). Photosynthesis and conductance of spring wheat ears: field response to free-air CO \(_2\) enrichment and limitations. *Plant Cell Environ.* 23, 917–929. doi: 10.1046/j.1365-3040.2000.00603.x

Xu, H., Qin, F., Du, F., Xu, R., Xu, Q., Tian, C., et al. (2009). Photosynthesis in different parts of a wheat plant. *J. Food Agric. Environ.* 7, 399–404.

Zhou, B., Serret, M. D., Elazab, A., Bort, J., Araus, J. L., Aranjuelo, I., et al. (2014). Wheat ear carbon assimilation and nitrogen remobilization contribute significantly to grain yield. *J. Integr. Plant Biol.* 58, 914–926. doi: 10.1111/jipb.12478

Zhu, C., Zhu, J., Liu, G., Zeng, Q., Xie, Z., Pang, J., et al. (2008). Elevated CO \(_2\) concentration enhances the role of the ear to the flag leaf in determining grain yield of wheat. *Photosynthetica* 46, 318–320. doi: 10.1007/s11099-008-0059-z

Zhu, C., Zhu, J., Liu, G., Zeng, Q., Xie, Z., Pang, J., et al. (2008). Elevated CO \(_2\) concentration enhances the role of the ear to the flag leaf in determining grain yield of wheat. *Photosynthetica* 46, 318–320. doi: 10.1007/s11099-008-0059-z

Zhu, C., Zhu, J., Liu, G., Xie, Z., Wang, H., et al. (2009). Elevated CO \(_2\) accelerates flag leaf senescence in wheat due to ear photosynthesis which causes greater ear nitrogen sink capacity and ear carbon sink limitation. *J. Exp. Bot.* 62, 391–399. doi: 10.1071/FB08269

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2020 Gómez, Vicente, Sanchez-Bragado, Jaureguy, Morcuende, Goicoechea and Aranjuelo. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.