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Influence of water stress on photosynthetic characteristics in barley plants under ambient and elevated CO_2 concentrations

A. ROBREDO[1], U. PÉREZ-LÓPEZ[1], M. LACUESTA[2], A. MENA-PETITE[1]* and A. MUÑOZ-RUEDA[1]

Departamento de Biología Vegetal y Ecología, Facultad de Ciencia y Tecnología,
Universidad del País Vasco/EHU, Apdo. 644, E-48080 Bilbao, Spain[1]
Departamento de Biología Vegetal y Ecología, Facultad de Farmacia, Universidad del País Vasco/EHU,
Apdo 450, E-01080 Vitoria-Gasteiz, Spain[2]

Abstract

We evaluated the combined effects of elevated CO_2 and water availability on photosynthesis in barley. Soil and plant water content decreased with water stress, but less under elevated CO_2 concentration (EC) compared with ambient CO_2 concentration (AC). During water stress, stomatal conductance, carboxylation rate, RuBP regeneration, and the rate of triose phosphate utilisation (TPU) were decreased but less when plants grew under EC. Drought treatments caused only a slight effect on maximum photochemical efficiency (variable to maximum fluorescence ratio, F_v/F_m), whereas the actual quantum yield (Φ_{PS2}), maximum electron transport rate (J_{max}) and photochemical quenching (qP) were decreased and the non photochemical quenching (NPQ) was enhanced. Under water deficit, the allocation of electrons to CO_2 assimilation was diminished by 49 % at AC and by 26 % at EC while the allocation to O_2 reduction was increased by 15 % at AC and by 12 % at EC.

Additional key words: climate change, drought, electron transport allocation, *Hordeum vulgare*, photochemical efficiency, quantum yield.

Introduction

It has been predicted that by the end of this century, atmospheric carbon dioxide concentration will amount to 700 μmol mol^{-1} (Fangmeier *et al.* 2000). Other consequence of the global change predicted might be increased drought notably in the Mediterranean area, where water is already an important factor limiting plant growth (Ruiz-Sanchez *et al.* 2007). Such changes in CO_2 concentration and water availability affect various physiological processes in plants, although the understanding of the interaction between these factors is still incomplete (Tezara *et al.* 2002 and references therein).

Barley has always been one of the world's most extensively cultivated crops and it has been reported that its yield will increase by 0.35 % per 1 μmol mol^{-1} increase in CO_2 (Manderscheid and Weigel 1995). It is extensively cultivated in the Mediterranean region (Lopes *et al.* 2004) and it seems to be, among the main temperate cereals, the best adapted to water shortage (Sánchez-Díaz *et al.* 2002). In spite of its economic importance relatively little work has been done on the effects of EC

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Abbreviations: AC - ambient CO_2 concentration; c_a - external CO_2 concentration; c_i - intercellular CO_2 concentration; EC - elevated CO_2 concentration (700 μmol mol^{-1}); ETR - the apparent total electron transport rate; Φ_{CO_2} - the apparent quantum yield of CO_2 fixation; Φ_{PS2} - actual quantum yield of PS 2; F_0, F'_0 - minimum fluorescence of dark- and light-adapted leaves; F_m, F'_m - maximum fluorescence of dark- and light-adapted leaves; F_s - steady-state fluorescence in light-adapted leaves; F_v/F_m - photochemical trapping efficiency in dark-adapted leaves; F'_v/F'_m - photochemical efficiency of PS 2 open centres; g_s - stomatal conductance; J_{max} - maximum electron transport rate; J_c - carboxylation electron transport; J_o - oxygenation electron transport; NPQ - non-photochemical quenching of fluorescence yield; P_N - net photosynthetic rate; P_{Nmax} - P_N at saturating CO_2 concentration; PPFD - photosynthetically active photon flux density; qP - photochemical quenching of fluorescence yield; R_D - respiration rate in the dark; R_L - respiration rate in the light; RSWC - relative soil water content; RWC - leaf relative water content; TPU - triose phosphate utilisation; V_{cmax} - maximum carboxylation rate; Γ_{CO_2} - carbon dioxide compensation point; ψ_o - leaf osmotic potential; ψ_w - leaf water potential.

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* Author for correspondence; fax: (+34) 946 0135 00; e-mail: amaia.mena@ehu.es

on barley plants (Kleemola *et al.* 1994, Hibberd *et al.* 1996, Bunce 2000, Sicher 2001, Kurasová *et al.* 2003), and never in relation with water stress. Only the interaction of EC and atmospheric humidity have been marginally studied in this species by Bunce (2000).

Elevated CO_2 increases photosynthetic rate and productivity, particularly in C_3 plants (Drake *et al.* 1997). Photosynthesis in C_3 plants responds to increased CO_2 concentrations because the present atmospheric concentration does not saturate Rubisco. Moreover, the oxidative carbon cycle (photorespiration) is inhibited by higher CO_2 concentration (Long and Drake 1992, Drake *et al.* 1997). However, these responses may be modified by other environmental factors such as water availability (Lawlor and Mitchell 1991, Robredo *et al.* 2007) or salinity (Pérez-López *et al.* 2009). Thus, studies are needed to explain the relationship between the stimulation of photosynthesis and the biochemical and physiological parameters that control overall net carbon assimilation, and the way that water stress can modulate the dynamics of the processes that constitute photosynthesis as a whole. Water deficit decreases photosynthetic capacity while this effect is delayed by EC (Robredo *et al.* 2007), but there is no agreement as to the nature of the underlying mechanisms (Habash *et al.* 1995, Lawlor 2002, Tezara *et al.* 2002). It has been suggested that most of the reduction in photosynthesis rate in response to water stress is attributable to stomatal closure; however, the decrease of leaf water potential also affects intrinsic photosynthetic capacity. The reduction in photosynthetic rate resulting from stomatal closure can be partially overcome by the EC concentration, but could the decrease in intrinsic photosynthetic capacity be offset by EC? Moreover, could the benefits that EC produces on photosynthesis under water stress be reduced if changes in the biochemistry and physiology of photosynthesis occur?

The main objectives of this study were *1*) to determine the effects of EC and drought on photosynthetic metabolism and related gas exchange properties, and the functionality of the photosynthetic apparatus in barley, and *2*) to assess whether exposure to EC allows barley plants to better withstand drought stress. To test these hypotheses, we evaluated gas exchange parameters and fluorescence characteristics under drought and well-watered conditions in barley plants exposed to AC and EC.

Materials and methods

Barley (*Hordeum vulgare* L. cv. Iranis) plants were grown in a mixture of *Perlite*:*Vermiculite* (3:1, v/v). Plants were grown in *Conviron E15* (*Conviron*, Manitoba, Canada) chambers under a 14-h photoperiod, the photosynthetic photon flux density (PPFD) 400 μmol m^{-2} s^{-1} (providing a total of 20 mol m^{-2} d^{-1}), day/night temperature of 24/20 ºC and relative humidity of 60/80 %. Light was supplied by a combination of incandescent bulbs and warm-white fluorescent lamps (*Sylvania F48T12 SHO*/*VHO*, *Sylvania*, Danvers, USA). Plants were grown either under ambient CO_2 concentration (AC*,* 350 μmol mol^{-1}) or under elevated CO_2 concentration (EC*,* 700 μmol mol^{-1}). An infrared gas analyzer coupled with a feedback control system controlled the injection of pure CO_2 gas, maintaining the required $[CO_2]$ within the chamber. To minimize the effects of intra-chamber environmental gradients, the plants were randomly repositioned within the chamber each week. The pots were watered three times a week with a complete Hoagland solution containing 20 mM nitrogen in the form of nitrate to ensure that a possible acclimation to elevated CO_2 was unrelated to nitrogen limitation. Drought was initiated when seedlings were 18 d old. Plants were divided into two groups, one of which was subjected to drought while the other received sufficient water to maintain soil water content close to pot capacity and served as the control group. The drought treatment was imposed by withholding water for 9, 13 and 16 d. Relative soil water content (RSWC), leaf water potential (ψ_w), leaf osmotic potential (ψ_o) and leaf relative water content (RWC) were determined as in Robredo *et al.* (2007).

Measurements of photosynthetic rate (P_N) and stomatal conductance (g_s) were done using a portable photosynthesis system *Li-Cor 6400* (Lincoln, NE, USA) as in Robredo *et al.* (2007) on intact leaves under saturating PPDF of 1200 μmol m^{-2} s^{-1} at 24 ºC and 60 % air humidity. Photosynthesis was initially induced at the growth CO_2 concentration (c_a), and then c_a was reduced stepwise to 60 μmol mol^{-1}. Thereafter the reference CO_2 concentration was increased to 80, 100, 150, 250, 350, 550, 700, 850, 1000, 1300, 1600 and 2000 μmol mol^{-1}. The c_i at each c_a was calculated using the equations of Von Caemmerer and Farquhar (1981). Photosynthetic-derived parameters such as carboxylation efficiency (CE), P_N at saturating CO_2 concentration (P_{Nmax}), respiration rate in the light (R_L), maximum *in vivo* electron transport rate (J_{max}), the maximum carboxylation velocity of Rubisco (V_{cmax}) and triose phosphate utilization (TPU) were calculated by the equations of Farquhar and Von Caemmerer (1982) with the *Photosyn Assistant Program* of Parsons and Ogstone (1997).

Chlorophyll *a* fluorescence was measured basically as described by González-Moro *et al.* (2003), using the *OS5-FL* modulated fluorometer (*Opti-Sciences*, Tyngsboro, USA). Next we determined the maximum quantum efficiency of PS 2 [$F_v/F_m = (F_m - F_0)/F_m$] (Schreiber *et al.* 1994). The actual quantum yield of PS 2 electron transport, $\Phi_{PS2} = (F'_m - F_s)/F'_m$. The coefficients of photochemical (qP) and non-photochemical (NPQ) quenching were calculated, as qP = $(F'_m - F_s)/(F'_m - F'_0)$ (Schindler and Lichtenthaler 1996) and NPQ = $(F_m - F'_m)/F'_m$ (Demmig-Adams *et al.* 1997). The apparent electron transport rate (ETR) was calculated as: ETR (Jt) = $\Phi_{PS2} \times$ PPFD × 0.84 × 0.5, where PPFD was 400 μmol m^{-2} s^{-1}, 0.84 was the coefficient of absorption of the leaves, and 0.5 was assumed as the fraction of the excitation energy distributed to PS 2.

Partitioning of total electron flow (Jt) between carboxylation (J_c) and oxygenation (J_o) was determined as in Epron *et al.* (1995): $J_t = J_c + J_o$, $Jc = 1/3\ [J_t + 8\ (P_N + R_D)]$, $Jo = 2/3\ [J_t - 4\ (P_N + R_D)]$.

At least three independent series of experiments were carried out for each time course of gas analysis and fluorescence parameter determinations. Data analyses were carried out using *SPSS* (Chicago, IL, USA) software package. Analysis of variance (two-ways) was tested for significance at $P \leq 0.05$. Differences between means were established using a Tukey test (Robredo *et al.* 2007).

Results

Soil water content (RSWC) was reduced by 88 % under AC and by 75 % under EC during the 16-d period of withholding water. Nearly similar reductions in soil water content (78 % under AC and 75 % under EC) were reached after 13 and 16 d, respectively. Thus, the rate of water use ($P \leq 0.01$) differed between the two CO_2 treatments, and RSWC diminished more slowly under EC. Application of the different watering regimes also decreased the RWC and midday ψ_w and ψ_o, and the effects of drought were similar after 13 (AC) and 16 d (EC) of withholding water (Table 1).

P_N and g_s were influenced by CO_2 ($P \leq 0.001$) and drought ($P \leq 0.001$) (Table 1). When P_N was measured at the growth CO_2 concentration, combining all measurement data on well-watered plants, the relative effect of CO_2 enrichment was an increase of 30 % (Table 1). Under water shortage, P_N was significantly ($P \leq 0.001$) higher (110 %) in EC compared to AC plants. Drought caused a 58 % reduction of P_N in EC-grown plants and 80 % in AC-grown ones (Table 1). The stomatal conductance of well-watered plants was greatly (40 %) decreased by growth under EC ($P \leq 0.001$). During the period of water stress g_s was similarly decreased (90 %) in both AC- and EC-grown plants (Table 1).

P_N plotted as a function of c_i and measured under saturating PPFD was greater in plants grown at EC at c_i higher than 150 μmol mol^{-1} (Fig. 1*B*), compared with those grown at AC (Fig. 1*A*). In well-watered plants, the P_{Nmax} and the CE of EC plants were increased by about 30 and 45 %, respectively, compared to AC-grown ones (Table 1). J_{max} (36 %), V_{cmax} (30 %) and TPU (38 %)

Table 1. Relative soil water content, RSWC [%]; leaf relative water content, RWC [%]; midday leaf water and osmotic potential, ψ_w, ψ_o [-MPa]; net photosynthetic rate, P_N [μmol(CO_2) m^{-2} s^{-1}]; stomatal conductance, g_s [mol(H_2O) m^{-2} s^{-1}]; net photosynthetic rate at saturating CO_2 concentration, P_{Nmax} [μmol(CO_2) m^{-2} s^{-1}]; carboxylation efficiency, CE [mol (CO_2) m^{-2} s^{-1}]; maximum electron transport rate, J_{max} [μmol(e^-) m^{-2} s^{-1}]; maximum carboxylation rate of Rubisco, V_{cmax} [μmol(CO_2) m^{-2} s^{-1}]; triose phosphate utilization, TPU [μmol m^{-2} s^{-1}]; respiration rate in the light, R_L [μmol(CO_2) m^{-2} s^{-1}]; carbon dioxide compensation point, Γ_{CO_2} [μmol(CO_2) mol^{-1}]; and apparent quantum yield of CO_2 assimilation, Φ_{CO_2} [μmol(CO_2) $mmol^{-1}$] of barley plants grown at AC and EC, and under well-watered conditions (D0) or after withholding water for 9 (D9), 13 (D13) or 16 (D16) d. D0 is the average value over all measurement dates. Means ± SE of at least three independent experiments, each replicated three-times. * refers to difference at $P \leq 0.05$; ** at $P \leq 0.01$; *** at $P \leq 0.001$.

Parameters	AC				EC						
	D0	D9	D13	D16	D0	D9	D13	D16	C	D	C×D
RSWC	100 ±0.00	31.3±4.50	21.9±2.90	11.4±1.90	100 ±0.00	39.5±3.20	31.4±5.50	25.3±2.80	**	***	*
RWC	97.7±0.50	92.2±2.60	75.7±3.10	57.7±4.60	95.5±0.50	93.2±1.50	90.7±1.60	79.6±1.90	***	***	***
ψ_w	0.23±0.02	0.90±0.09	1.35±0.10	1.64±0.09	0.34±0.03	0.61±0.06	0.94±0.10	1.30±0.08	**	***	***
ψ_o	1.31±0.02	1.57±0.11	1.80±0.07	2.94±0.03	1.42±0.06	1.26±0.04	1.42±0.07	1.72±0.08	***	***	***
P_N	12.1±0.89	6.70±0.45	3.30±0.68	2.50±1.22	15.7±1.27	15.7±1.27	9.00±0.42	6.60±1.04	***	***	***
g_s	0.34±0.05	0.12±0.01	0.04±0.01	0.03±0.00	0.20±0.01	0.07±0.01	0.03±0.00	0.02±0.00	***	***	***
P_{Nmax}	22.4±2.71	16.5±0.43	7.40±0.84	6.50±1.33	28.8±1.80	22.7±1.18	19.1±3.18	16.7±2.26	***	***	n.s.
CE	0.06±0.00	0.06±0.00	0.03±0.00	0.03±0.00	0.09±0.01	0.07±0.00	0.07±0.01	0.06±0.00	***	***	n.s.
J_{max}	111 ±2.40	93.8±4.60	49.6±12.4	25.7±11.7	151 ±3.50	124 ±9.50	104 ±14.1	104 ±7.60	***	***	n.s.
V_{cmax}	37.6±1.30	35.9±2.30	21.0±2.60	12.6±1.60	48.8±1.40	43.8±3.5	40.2±1.90	37.3±1.50	***	***	n.s.
TPU	8.13±0.21	5.69±0.70	4.41±0.77	3.87±0.77	11.2±0.27	9.84±1.63	7.09±1.04	6.85±0.60	***	***	n.s.
R_L	1.40±0.30	1.80±0.30	2.00±0.90	2.80±0.90	1.30±0.40	2.50±0.30	2.50±0.30	2.20±0.70	***	***	n.s.
Γ_{CO2}	58.3±1.60	63.9±3.90	83.6±14.7	117 ±48.6	53.9±2.30	73.0±5.70	72.7±2.90	77.8±2.90	n.s.	**	n.s.
Φ_{CO2}	49.0±16.4	36.0±11.4	32.5±11.6	16.7±2.70	86.0±17.5	69.8±8.40	60.0±8.00	42.0±10.0	n.s.	*	n.s.

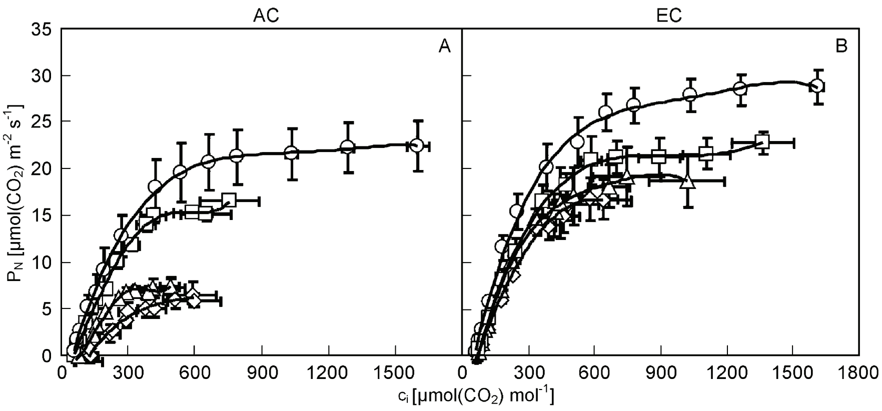

Fig. 1. Response of net photosynthetic CO_2 assimilation (P_N) to intercellular CO_2 concentration (c_i) of barley plants grown at ambient (*A*) and elevated (*B*) [CO_2] and subjected to well-watered conditions (*circles*) or 9 (*squares*), 13 (*triangles*) and 16 d (*diamonds*) of water stress. Means ± SE of at least three independent experiments, each replicated three-times.

were also significantly increased ($P \leq 0.001$) by EC. Light respiration and the compensation CO_2 concentration seemed not to be statistically modified in well-watered EC-grown plants (Table 1). Under water stress R_L was increased by nearly 100 % in both AC and EC grown plants, whereas Γ_{CO2} was enhanced by nearly 100 %, at the end of drought period, in AC plants and by 50 % in EC ones (Table 1).

Water shortage caused a greater reduction of P_{Nmax} in AC (72 %) than in EC plants (42 %) after 16 d withholding water. Similarly, the effect of drought on CE was lower (36 %) in EC than in AC plants. The leaf water potential was -0.3 MPa in well-watered plants, -0.9 MPa at mild water stress and -1.3 MPa at moderate water stress. All photosynthetic parameters were significantly affected ($P \leq 0.01$) by water stress but the effect was also higher under AC (Fig. 2). Thus, when leaf water potential was -0.9 MPa (9 d of drought in AC plants *versus* 13 d in EC ones), P_{Nmax}, J_{max}, V_{cmax} and TPU (Fig. 2) were by 16, 11, 12 and 25 %, respectively, higher in EC than in AC grown plants. When water potential dropped to -1.3 MPa (after 13 d of drought in AC plants *versus* 16 d in EC ones), the same parameters were higher by 126, 110, 78 and 55 % in EC than in AC grown plants. That is,

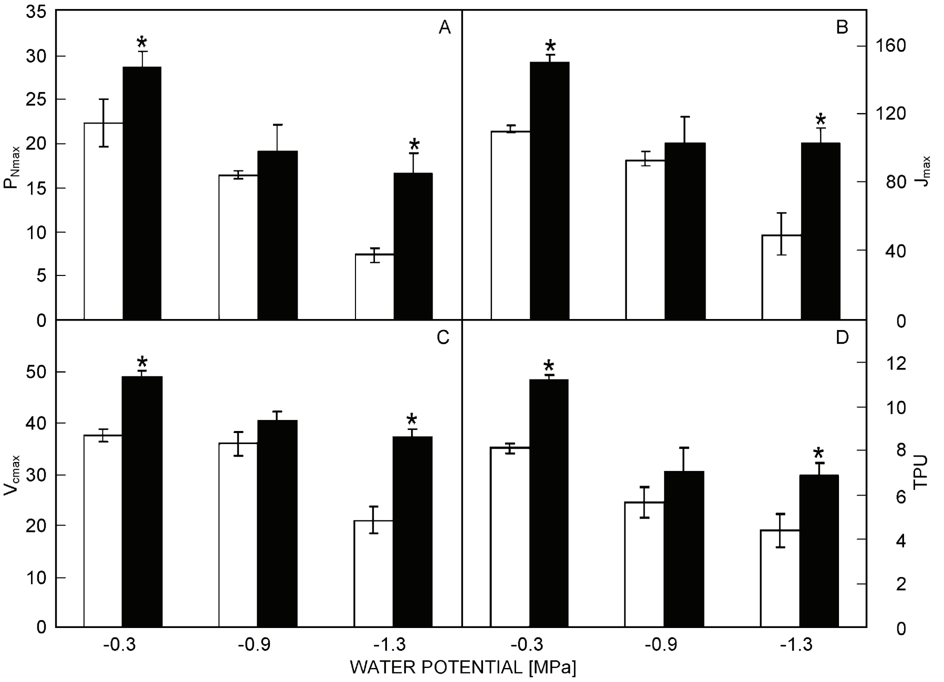

Fig. 2. Effects of midday water potential on P_{Nmax} [μmol(CO_2) m^{-2} s^{-1}] (*A*), J_{max} [μmol(e^-) m^{-2} s^{-1}] (*B*), V_{cmax} [μmol(CO_2) m^{-2} s^{-1}] (*C*) and TPU [μmol m^{-2} s^{-1}] (*D*) in barley plants grown at ambient (*white bars*) and elevated (*black bars*) CO_2 concentrations well-watered (-0.3 MPa), or after mild (-0.9 MPa) or moderate (-1.3 MPa) water stress. * indicates significant differences at $P \leq 0.05$ between CO_2 treatments at a corresponding water potential.

Table 2. Chlorophyll *a* fluorescence parameters: photochemical trapping efficiency in dark-adapted fluorescence, F_v/F_m; actual quantum yield of PS 2, Φ_{PS2}; coefficient of photochemical quenching of fluorescence, qP; non-photochemical quenching of fluorescence yield, NPQ and partitioning of total electron flow, ETR [μmol(e-) m^{-2} s^{-1}] between carboxylation, J_c and oxygenation, J_o determined as Epron *et al.* (1995), in barley plants. Plant growth conditions and statistical analysis as in Table 1.

Parameters	AC				EC						
	D0	D9	D13	D16	D0	D9	D13	D16	C	D	C×D
F_v/F_m	0.82±0.00	0.81±0.01	0.74±0.03	0.69±0.10	0.81±0.01	0.81±0.01	0.80±0.01	0.74±0.04	*	***	**
Φ_{PS2}	0.61±0.01	0.54±0.07	0.46±0.05	0.39±0.05	0.67±0.02	0.61±0.02	0.52±0,02	0.50±0.02	n.s.	***	**
qP	0.91±0.01	0.82±0.08	0.69±0.06	0.55±0.03	0.89±0.02	0.83±0.03	0.76±0.03	0.71±0.05	n.s.	**	n.s.
NPQ	0.36±0.04	0.49±0.13	0.58±0.11	0.23±0.05	0.38±0.05	0.45±0.05	0.46±0.12	0.22±0.01	n.s.	n.s.	n.s.
ETR	101 ±2.00	90.0±12.0	78.0±8.00	65.0±9.00	112 ±4.00	95.0±5.00	87.0±3.00	83.0±4.00	n.s.	***	**
J_c	69.8	52.7	40.1	35.7	83.0	67.7	59.7	51.1			
J_o	31.2	37.3	37.9	29.3	29.0	27.3	27.3	31.9			

when plants with the same water potential (-1.30 MPa) were compared (Fig. 2), the drought effect on all the parameters obtained from the P_N/c_i curves were from 10 to 50 % less affected in EC than in AC plants.

The F_v/F_m ratio was not affected by elevated CO_2 in well-irrigated plants and was always above 0.80, the value considered typical of healthy plants. Under water stress, AC and EC plants experienced a 16 and 8 % reduction in F_v/F_m, respectively, after 16 d of drought (Table 2). No significant differences between the two CO_2 concentrations were observed in the Φ_{PS2} in plants not subjected to drought. After 16 d of withholding water, AC plants decreased their Φ_{PS2} by 36 %, while the same period of water shortage only caused a 25 % reduction in EC plants. The Φ_{PS2} takes into account two parameters: the efficiency of excitation energy capture by open PS 2 reaction centres (F'_v/F'_m) and the variable proportion of open PS 2 reaction centres (qP). The qP was not significantly different in leaves of EC than in AC well-watered plants. On the contrary, a 12 % increase in F'_v/F'_m was registered in EC plants compared to their AC counterparts (data not shown). When plants were subjected to water restriction, the qP was reduced by 40 % in AC plants and by 20 % in EC plants, while F'_v/F'_m was not affected by water stress in AC plants whereas it was reduced between 2 and 9 % in EC grown plants (data not shown). Thus, the Φ_{PS2} drought-mediated changes depended strongly on qP.

The NPQ was not significantly affected (< 8 %) by CO_2 concentration in well-watered plants. However, the high variability of the data made it impossible to discern a clear trend in NPQ when plants were enduring drought. NPQ increased by 60 % in AC plants and only by 20 % in EC ones, as drought progressed until 13 d, and afterwards dropped (Table 2). The apparent photosynthetic linear electron transport rates through PS 2 (J_t and J_c) were increased by 11 and 19 %, respectively, for plants grown and measured at elevated CO_2, compared to those grown and measured at AC (Table 2). Elevated CO_2 reduced (by 5 %) the allocation of electron transport to photorespiration (J_o, Table 2). After 16 d of water deprivation, J_t was reduced by 36 % in AC plants, and by 26 % under EC conditions. The flux to carboxylation (J_c) was reduced by 49 % in AC-grown plants and by 38 % in EC-grown ones. Consequently, the percentage of oxygenation flow (J_o/J_t) was increased by drought in both CO_2 concentrations (Table 2).

The Φ_{CO2} was positively correlated with Φ_{PS2} under both CO_2 concentrations (AC: $r^2 = 0.956$; EC: $r^2 = 0.904$) and was also influenced by the water regime. For similar photochemical efficiency, the Φ_{CO2} was higher in plants grown in elevated CO_2, confirming that under AC and water stress, a large part of electron transport was allocated to non carboxylating reactions (Table 2). Water stress caused a more severe effect on photosynthesis quantum yield (65 and 51 % inhibition) than on photochemical efficiency (36 and 25 % reduction) under AC and EC concentrations, respectively.

Discussion

As reported in a previous work (Robredo *et al.* 2007), soil water content was less depleted under EC compared to AC, which was in accordance with the lower rates of transpiration (Robredo *et al.* 2007). Similar soil conservation has been observed by Rogers *et al.* (1984) and Vu *et al.* (1998). EC had no significant effect on the water relations of irrigated plants, whereas RWC, ψ_w and ψ_o were higher at elevated compared to AC during the entire drought period. Thus, plant water stress developed more slowly due to a slower rate of soil water depletion. This better water status also appeared to be the result of stomatal control since no osmotic adjustment was observed in previous experiments (Robredo *et al.* 2007), despite the increased rates of P_N in EC (Table 1). Similarly, Picon *et al.* (1996) and Polley *et al.* (1999) observed no osmotic adjustment under elevated CO_2.

The decline in P_N with decreasing ψ_w was correlated with a reduction in g_s (Table 1, Melgar *et al.* 2008), which reduced diffusion of CO_2 into the leaf (c_i decreased; Robredo *et al.* 2007). However, in spite of the lower g_s at EC, P_N was higher than at AC. The higher CO_2 concentration enabled plants to offset the restriction imposed by stomata (Robredo *et al.* 2007). P_N was maintained for longer period at EC when plants were water deprived; indeed, the stimulation of P_N by EC was even greater under drought compared to well-watered plants (Table 1).

By analysing the P_N/c_i curves (Fig. 1), we were able to deduce the relative importance of stomatal and non stomatal limitations to photosynthesis under both CO_2 concentrations, as well as the response of photosynthetic biochemical parameters to CO_2 and water availability. From curves measured at saturating irradiance, the P_{Nmax}, practically saturated at $c_i \geq 600$ µmol mol^{-1} in AC-grown plants, while in EC-grown P_{Nmax} was satured at $c_i \geq 900$ µmol mol^{-1}. This saturation value is associated with the inhibition imposed by RuBP regeneration (Lawlor 2002). However, the *in vivo* activity of Rubisco (V_{cmax}) and the capacity for regeneration of RuBP (J_{max}) were increased at EC. Therefore, the increased capacity of carbon fixation in plants grown at EC is in part due to a reduction in J_O (Table 2), but also due to an increase in Rubisco activity as supported by the increase in CE (Table 1) calculated from *in vivo* P_N/c_i curves (Von Caemmerer and Farquhar 1981, Habash *et al.* 1995). This increase in the capacity of photosynthesis under EC is also a result of an increase in electron transport (Table 1).

We observed a marked decrease in CO_2 assimilation rates at all c_i values following water stress under both CO_2 concentrations. Both P_{Nmax} and CE (Table 1) declined markedly and significantly with decreasing ψ_w, as was also observed by Tezara *et al.* (2002) in sunflower. Low values of J_{max} (Fig. 2*B*) would indicate a reduced rate of RuBP regeneration and the reduced values of V_{cmax} (Fig. 2*C)* would be related to decreased Rubisco activity as postulated by Lopes *et al.* (2004) and Centritto (2005). The higher effect of water stress on J_{max} than on V_{cmax} indicates that the regeneration of RuBP was more affected by drought than the RuBP carboxylase activity under both CO_2 concentrations. On the other hand, when plants were subjected to drought, photosynthate translocation (Fig. 2*D*) was markedly reduced in both CO_2 concentrations indicating that drought affected the allocation of the carbon assimilated. Therefore, water stress induced marked reduction in the *in vivo* parameters describing plant photosynthetic capacity (metabolic impairment) but there were interactions between EC and water stress, with these parameters being less affected under EC, even when ψ_w dropped to similar values (Fig. 2).

Revision of the literature shows a tendency to an increase in R_D as a consequence EC, but only few reported differences were significant (Ulman *et al.* 2000). Our findings showed that as soil drying progressed, R_D increased (Table 1). This enhancement of R_D could be related to increased mitochondrial consumption of sugar reserves under water stress. The CO_2 compensation point also increased as water stress developed.

On the other hand, plants have the ability to adjust their biochemical and photochemical activities minimizing any imbalance of cellular homeostasis (Pérez-López *et al.* 2009). Under water stress, the deprivation of CO_2 at the chloroplast level by stomata closure could enhance the sensitivity of the photosynthetic apparatus to photoinhibition (Escalona *et al.* 1999). It has been observed that as drought stress rises, a significant alteration occurs in all fluorescence parameters (Mena-Petite *et al.* 2005). In our experiments, photochemical efficiency was only slightly reduced by water stress and only after 16 d of water deprivation, and this injury was delayed when plants were grown at EC. Lawlor and Cornic (2002) and Centritto (2005) also found that F_v/F_m was decreased only at severe water deficit, suggesting that the PS 2 activity is resistant to water deficit (Tezara *et al.* 2008). On the other hand, a 10 % increase in Φ_{PS2} was observed in well-watered EC plants. This increase was due to an increase (12 %) in the efficiency of open centres (F'_v/F'_m) whereas only a slight reduction (2 %) was observed in the proportion of open PS 2 reaction centres (qP). This finding is in accordance with results by Long and Drake (1991) and Habash *et al.* (1995), who observed an increase in the Φ_{PS2} under elevated CO_2. However, Φ_{PS2} was reduced by water stress. We found a close relationship between the decrease in quantum yield and the reduction in qP with decreasing ψ_w in plants grown at AC (Table 2). The decrease of qP as the water deficit progressed indicated a higher reduction state of primary acceptors in stressed plants. The reduction in qP and Φ_{PS2} and the simultaneous lack of change in F_v/F_m could be associated with a down-regulation of PS 2 during water stress, representing a photoprotective mechanism to match the rate of photochemistry with the consumption of ATP and NADPH. The Φ_{PS2} of EC plants was 35 % less affected by water stress. The increase in dissipation of the excitation energy of PS 2 through photochemical processes (qP) in water-stressed plants when grown under elevated CO_2 may thus decrease the risk of photoinhibition.

The NPQ was not significantly affected by CO_2 concentration in well-watered plants, indicating that no changes in energy dissipation by heat occurred in plants grown under EC. When biochemical and photochemical processes are disrupted by water deprivation, NPQ increased contributing to non-photochemical dissipation of the energy (Lawlor and Cornic 2002), and thus preventing injury of the thylakoids. The higher P_N under EC acts as a major sink for ATP and NADPH lowering the need for thermal dissipation of energy and thus the increase of NPQ is less (Table 2).

Although water deficiency decreased the maximal rate of electron flux (Table 1), as expected from the decrease in P_N (Tezara *et al.* 2002), the proportion of total

ETR (J_t) to photorespiration increased in response to drought under both CO_2 concentrations (Table 2). The possible reduced sink strength caused by drought may cause feedback inhibition of photosynthesis (Lambers *et al.* 1998). The lower reduction of J_t than P_N due to water deficit may be due to higher electron flow to other acceptors, as suggested by Lawlor and Cornic (2002). In fact, J_c was more affected that J_o (Table 2), and J_o/J_t was increased. Similar behaviour has been observed in sunflower under water deficit (Tezara *et al.* 2008). The maintenance of electron flow to oxygenation reactions, such as photorespiration or other additional sinks such as Mehler reaction, may minimize or prevent damage to the PS 2 under water stress and might explain the low decrease in F_v/F_m of dark-adapted leaves, indicating no permanent photoinhibitory damage by drought to the photosynthetic machinery. From our results, an increase in the ratio Φ_{PS2}/Φ_{CO2} following the water stress treatment can be inferred. This increase indicates that electron flow can be sustained by additional electron consumption in pathways other than the Calvin cycle.

EC stimulated J_c to a greater extent than it suppressed J_o, increasing J_t (ETR) (Table 2). The decreased allocation of electron transport to photorespiration (J_o/J_t) by EC (Table 2) increased the flow of electrons to Rubisco carboxylation (J_c) changing the balance between carbon fixation and photorespiration (2.23 in AC *versus* 2.85 in EC well-watered plants, and 1.21 in AC *versus* 1.60 in EC plants after 16 d withholding water) as a result of changes in the kinetics of Rubisco (Habash *et al.* 1995). This fact could explain the lesser effect of drought on qP, with less reduction of the photochemical intermediates and mitigation of the effect of water stress on the photosynthetic machinery (photoinhibition), as evidenced by the higher values of F_v/F_m and Φ_{PS2} in EC-grown plants (Table 2). Similar results were found by Zhang and Dang (2005), confirming the theory that the stimulation of photosynthesis by elevated CO_2 occurs through the promotion of Rubisco carboxylation and depression of photorespiration (Long and Drake 1992, Sage 1994, Drake *et al.* 1997).

In conclusion, this study reports data indicating that EC stimulates photosynthesis, raising total linear electron transport and enhancing the utilization of ATP and NADPH. Moreover, plants grown at EC show a higher capacity for RuBP regeneration as well as an increase in the amount of potential activity of Rubisco, supported by the increase in carboxylation efficiency (P_N/c_i curves). In addition, EC plants exhibit a higher quantum yield of photosynthesis for similar photochemical efficiency (Φ_{CO2}/Φ_{PS2}). On the other hand, the large reduction of photosynthetic rates in water-stressed plants may be due not only to stomatal limitation but also to metabolic impairment as deduced from reduced photochemical efficiency. Furthermore, these findings show the biochemical and physiological mechanisms by which EC offsets the risk of damage to the photochemical apparatus, allowing plants to better withstand soil water deficit under elevated CO_2 concentration.

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