Effects of soil drought and atmospheric humidity on yield, gas exchange, and stable carbon isotope composition of barley

M. SÁNCHEZ-DÍAZ*, J.L. GARCÍA*, M.C. ANTOLÍN*, and J.L. ARAUS**

*Departamento de Fisiología Vegetal, Universidad de Navarra, c/Irunlarrea s/n, E-31008 Pamplona, Spain**
*Unitat de Fisiologia Vegetal, Departament de Biologia Vegetal, Facultat de Biologia, Universitat de Barcelona, 08028 Barcelona, Spain***

Abstract

The combined effects of water status, vapour pressure deficit (VPD), and elevated temperature from heading to maturity were studied in barley. Plants growing at high VPD, either under well-watered or water deficit conditions, had higher grain yield and grain filling rate than plants growing at low VPD. By contrast, water stress decreased grain yield and individual grain dry matter at any VPD. Water regime and to a lesser extent VPD affected $\delta^{13}C$ of plant parts sampled at mid-grain filling and maturity. The differences between treatments were maximal in mature grains, where high VPD increased $\delta^{13}C$ for both water regimes. However, the total amount of water used by the plant during grain filling did not change as response to a higher VPD whereas transpiration efficiency (TE) decreased. The net photosynthetic rate (P_N) of the flag leaves decreased significantly under water stress at both VPD regimes. However, P_N of the ears was higher at high VPD than at low VPD, and did not decrease as response to water stress. The higher correlation of grain yield with P_N of the ear compared with that of the flag leaf support the role of ear as the main photosynthetic organ during grain filling under water deficit and high VPD. The deleterious effects of combined moderately high temperature and drought on yield were attenuated at high VPD.

Additional key words: CO_2 balance; *Hordeum vulgare*; transpiration efficiency; water stress; water use efficiency.

Introduction

Mediterranean climate is characterised by hot, dry summers, coupled with high vapour pressure deficit (VPD). These multiple stresses occur during the critical period of grain filling, therefore affecting productivity of cereals. Under water-limited conditions, any improvement in either water use efficiency of photosynthesis, WUE_{ph} (the ratio of CO_2 assimilation to transpiration) or transpiration efficiency (TE, dry matter produced per unit of water transpired) should result in higher biomass if the total water used is similar (Turner 1993). The carbon isotope composition ($\delta^{13}C$) of plant dry matter (DM) has been proposed as a selection criterion because it provides information on long-term TE of plants (Farquhar and Richards 1984, Turner 1993) or its components (Behboudian *et al.* 2000).

High temperature is other environmental factor that affects considerably grain yield of cereals. Majority of the reduction in grain yield produced by high temperature during grain filling has been attributed to sink limitation given the similarities between the declines in grain number, grain yield, and harvest index (Ferris *et al.* 1998). Indeed, under high temperature the ability of cereal grains to sustain its growth rate is important to counterbalance the reduced duration of grain filling associated with high temperature (Wardlaw and Moncur 1995).

Among the main temperate cereals, barley is the best adapted to the severe water regime characterising semi-arid Mediterranean environments. This study contains original information on the combined effect of water status and vapour pressure deficit (VPD) on grain yield and related photosynthetic, WUE, and TE parameters in barley. Whereas there is abundant literature on the separate

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Fax (34 948) 425649, e-mail: msanchez@unav.es
Abbreviations: ALDM = areal leaf dry mass; DM = dry matter; $\delta^{13}C$ = stable carbon isotope composition; P_N = net photosynthetic rate; PPFD = photosynthetic photon flux density; R_D = dark respiration rate; RH = relative humidity; RWC = relative water content; TE = transpiration efficiency; VPD = vapour pressure deficit; WUE_{ph} = water use efficiency of photosynthesis; Ψ_w = water potential.
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effects of water stress or VPD on yield and its agronomic components (Morgan and Riggs 1981, Kobata *et al.* 1992, Mogensen 1992, Savin and Nicolas 1996), the interaction between these factors has been less studied. By much more scarce are the reports including the effect of these factors on gas exchange and WUE of the ear and the flag leaf. The ear is one of the main photosynthetic organs providing assimilates during grain filling, particularly in Mediterranean environments, where drought at the end of the crop cycle is common (Blum 1985, Araus *et al.* 1993, Bort *et al.* 1994). However, most studies concerning responses of photosynthetic organs to abiotic stresses have been performed on leaves alone.

Therefore, the objective of this work was to study the combined effect of water deficit and different VPD on barley plants growing at moderately high temperature during grain filling. Yield and its components, grain growth parameters, TE, CO_2 exchange, and $\delta^{13}C$ were investigated under controlled conditions.

Materials and methods

Growth conditions: Seeds of barley (*Hordeum vulgare* L. cv. Camelot) were surface disinfected and germinated on wet filter paper in Petri dishes. Seedlings were planted in 25×10 cm pots (1 600 cm^3 of volume) containing a mixture of *perlite* : *vermiculite* (1 : 1, v/v) (1 plant per pot). Plants were irrigated with Hoagland's nutrient solution and were maintained adequately irrigated until awn emergence (the soil water content was approximately 0.35-0.40 m^3 m^{-3}).

Initially, plants were grown in a greenhouse at 20/10 °C, 60/80 % RH (day/night) (equivalent to a water vapour pressure deficit, VPD, of 0.9 kPa during day) and 18-h photoperiod with natural daylight supplemented with fluorescent lamps (*Sylvania DECOR 183*, *Professional-58W*, München, Germany), providing a minimum photosynthetic photon flux density (PPFD) of 300-400 µmol m^{-2} s^{-1}.

When awn emergence was reached (Zadoks scale 51; Zadoks *et al.* 1974), plants were transferred to temperature-controlled chambers with a day/night temperature regime of 30/20 °C and an 18-h photoperiod of 400 µmol m^{-2} s^{-1} PPFD at ear level provided by fluorescent lamps (*Phillips TLD 58W-83*, Eindhoven, the Netherlands). Plants were separated in two controlled chambers having different VPD. Low-VPD plants grew at VPD equivalent to 0.9 kPa during day, whereas high-VPD plants grew at VPD equivalent to 2.1 kPa during day. In both treatments, night VPD was 0.5 kPa (RH 80 %).

Within each VPD-controlled chamber, plants were subdivided into two groups: (*1*) control well-watered plants that were replenished every day with the water lost by transpiration, and (*2*) plants subjected to water stress until maturity (Zadoks scale 92) that were supplied with a fraction of water transpired by controls. The amount of water supplied to the stressed plants was calculated on the basis of the average water lost by transpiration in irrigated control plants (T_c), the water transpired from each droughted plant (T_d), and a drought factor (F), calculated as: water supply = (2 F T_c) − T_d after Baigorri *et al.* (1999). The drought factor (F) corresponds to the desired equilibrium ratio of T_d/T_c, and was fixed as 1/3. Transpiration was measured by weighting the pots every day at the beginning of photoperiod. Direct evaporation from the pots was minimised by placing a 2-cm layer of white sand on the soil surface and by sealing the bottom of each pot with a plastic bag. Total transpiration per plant was calculated as the sum of water lost by transpiration during treatments.

Plant water status: All measurements were made at the mid-grain filling period (Zadoks scale 75). Midday leaf water potential (Ψ_w) was measured with a pressure chamber, and the midday leaf relative water content (RWC) was calculated as described by Weatherley (1970).

Gas exchange measurements and leaf characteristics: Net photosynthetic rate (P_N) was measured on attached ears and flag leaves at the mid-grain filling period (Zadoks scale 75) using a portable infra-red gas analyser (*LCA* model, *Analytical Development Co.*, Hoddesdon, UK) operated in the differential mode. Air of known CO_2 concentration (350 µmol mol^{-1}) and the corresponding VPD (2.1 or 0.9 kPa) was supplied at a constant flow (400 cm^3 s^{-1}) into the leaf chamber. Plant part (flag leaf or ear) temperature was 30 °C as monitored with a copper-constantan thermocouple attached to the underside of each organ. PPFD was 800 µmol m^{-2} s^{-1} (saturation with photons) as measured with a quantum sensor. Further, dark respiration rate (R_D) was determined after 20 min of darkness. Gas exchange parameters were calculated according to Caemmerer and Farquhar (1981) and either one side (flag leaf) or a prism area (ear) was considered, respectively.

Areal leaf dry mass (ALDM) was calculated as the leaf DM per unit leaf area. Chlorophyll content was determined spectrophotometrically in fresh samples of the same leaves used in gas exchange measurements, in acetone (80 %, v/v) extract (Šesták *et al.* 1971).

Transpiration efficiency (TE) and yield: TE was calculated at maturity (Zadoks scale 92) as the ratio of DM produced during treatments in the whole plant (TE_p) or the grain yield (TE_y) to the water transpired in each pot (Hubick and Farquhar 1989). Plant dry matter was determined after drying at 70 °C for two days.

Harvest maturity was defined as that stage when grain moisture declined naturally to 15 %. The duration of the grain-filling period was calculated as the time between anthesis and physiological maturity. The grain-filling rate was then estimated by fitting a linear regression model

(Loss *et al.* 1989). In addition, total number of ears per plant as well as number of florets and grains per ear and individual grain mass were evaluated at maturity.

Stable carbon isotope analysis: Samples were taken from leaves, awns, and grains at two stages, mid-grain filling (Zadoks scale 75) and grain maturity (Zadoks scale 92). Plant material was then ground and the $^{13}C/^{12}C$ ratio was determined by mass spectrometry as described by Bort *et al.* (1998). Samples of 0.5-0.7 mg were used. Results for carbon isotope composition were expressed as $\delta^{13}C$ values, where:

$$\delta^{13}C\ [‰] = [(R_{sample}/R_{standard}) - 1] \times 1\ 000,$$

R being the $^{13}C/^{12}C$ ratio. A secondary standard calibrated against PeeDee Belemnite (PDB) carbonate was used for comparison. Replicate samples differed in less than 0.10 ‰.

Statistical analysis: Analysis of variance (*ANOVA*) was performed to partition the variance into the main effects and the interaction between VPD and soil moisture (Sokal and Rohlf 1979). Means ± SE ($n = 10$) were calculated, and when F ratio was significant, least significant differences (LSD) were evaluated by the Tukey's t-test.

Results

Total transpiration rate and dry matter (DM) production decreased significantly with water deficit under both vapour pressure deficit (VPD) regimes, whereas transpiration efficiency on the whole plant basis (TE_p) increased as response to water deficit, especially at high VPD (Table 1). In addition, water deficit and VPD decreased significantly the grain yield. However, plants growing at high VPD exhibited higher grain yield than plants growing at low VPD (Table 1). Therefore, at high VPD plants had always a higher TE calculated on grain yield basis (TE_y).

The number of ears per plant was not affected by water status at any VPD. However, at low VPD the number of sterile ears (fruitless ears) increased, especially under water deficit (Table 2). Ear sterility increased under water deficit in both VPD regimes but was higher at low VPD. There were no differences in the number of grains per ear, but individual grain DM decreased significantly in drought-affected plants at any VPD.

Table 1. Total transpiration rate, transpiration efficiency of whole plant (TE_p), TE of grain yield (TE_y), total dry matter (DM) produced during treatments, and grain yield obtained in barley plants grown at moderately high temperature (30/20 °C, d/n) in combination with low (0.9 kPa) or high VPD (2.1 kPa) under well watered and water deficit conditions. Means of 10 plants. Comparisons between means were made with the Student's t-test. Within each column, means followed by the same letter are not significantly different (p>0.05). NS, *, **, and *** mean non-significant or significant at 5, 1, and 0.1 % probability levels, respectively.

Treatment		Total transpiration [kg plant^{-1}]	TE_p [g(DM) kg^{-1}(H_2O)]	TE_y [g(grain) kg^{-1}]	Total DM [g plant^{-1}]	Grain yield [g plant^{-1}]
Low VPD	Well-watered	1.77 a	3.03 c	0.65 c	5.20 ab	1.21 b
	Water deficit	0.65 b	4.29 b	0.78 bc	2.74 c	0.30 c
High VPD	Well-watered	1.96 a	3.02 c	1.16 ab	6.22 a	1.90 a
	Water deficit	0.75 b	5.23 a	1.56 a	3.84 bc	1.12 b
VPD		NS	NS	***	NS	***
Soil water		***	***	NS	***	***
Interaction		NS	NS	NS	NS	NS

Table 2. Yield components and percentage of ear sterility of barley plants grown at moderately high temperature and different VPD under well watered and water deficit conditions. Means of ten plants. Levels of significance as for Table 1.

Treatment		Ear number [plant^{-1}]	Sterile ears [plant^{-1}]	Ear sterility [% aborted ears]	Grain number [ear^{-1}]	Individual grain DM [mg]
Low VPD	Well-watered	4.0 a	1.8 b	41.9 b	14.9 a	40.6 a
	Water deficit	4.3 a	2.7 a	62.1 a	12.9 a	30.5 b
High VPD	Well-watered	4.8 a	0.9 c	16.0 c	12.8 a	42.8 a
	Water deficit	4.3 a	1.6 b	37.2 b	14.3 a	33.5 b
VPD		NS	***	***	NS	NS
Soil water		NS	**	***	NS	***
Interaction		NS	NS	NS	NS	NS

Table 3. Grain-filling rate and grain-filling duration of barley plants grown at moderately high temperature and different VPD under well watered and water deficit conditions. Means of ten plants. Levels of significance as for Table 1.

Treatment		Grain-filling rate [mg d^{-1}]	Grain-filling duration [d]
Low VPD	Well-watered	2.34 b	19.3 a
	Water deficit	1.89 c	16.5 c
High VPD	Well-watered	2.74 a	17.8 b
	Water deficit	2.42 ab	16.2 c
VPD		***	*
Soil water		**	***
Interaction		NS	NS

Table 4. Carbon isotope composition ($\delta^{13}C$) of different plant parts of barley plants grown at moderately high temperature and different VPD under well watered and water deficit conditions. Samples were obtained at two grain-filling stages: mid-grain filling (Zadoks scale 75; Zadoks *et al.* 1974) and physiological maturity (Zadoks scale 92). Each value is the result of a single analysis performed in a pool of 10 samples.

	Treatment		Flag leaf [‰]	Awns [‰]	Grain [‰]
Mid-grain filling	Low VPD	Well-watered	−31.75	−31.66	−
		Water deficit	−30.91	−30.85	−
	High VPD	Well-watered	−31.01	−31.32	−
		Water deficit	−30.04	−30.31	−
Physiological maturity	Low VPD	Well-watered	−30.31	−31.24	−32.16
		Water deficit	−30.29	−30.42	−31.19
	High VPD	Well-watered	−30.90	−31.22	−31.50
		Water deficit	−30.59	−29.36	−28.79

Table 5. Midday leaf water potential (Ψ_w), relative water content (RWC), areal leaf dry mass (ALDM), and leaf chlorophyll content of barley plants grown at moderately high temperature and different VPD under well-watered and water deficit conditions. Measurements were made at the mid-grain filling period (Zadoks scale 75). Means of 20 measurements. Levels of significance as for Table 1.

Treatment		Leaf Ψ_w [MPa]	Leaf RWC [%]	ALDM [g m^{-2}]	Chlorophyll [g kg^{-1}(DM)]
Low VPD	Well-watered	−1.7 a	93.3 a	45.9 b	9.6 a
	Water deficit	−1.8 a	90.1 b	45.5 b	8.4 b
High VPD	Well-watered	−1.8 a	91.4 ab	51.3 a	8.2 b
	Water deficit	−2.3 b	80.7 c	51.5 a	7.5 b
VPD		***	***	***	**
Soil water		***	***	NS	*
Interaction		*	***	NS	NS

Water deficit and VPD affected grain growth parameters differently (Table 3). High VPD increased grain-filling rate. However, soil water status showed no effect on grain filling rate when plants grew at high VPD, whereas this rate decreased significantly in low-VPD plants in response to water stress. On the other hand, grain-filling duration decreased in both VPD regimes under water stress, whereas the effect of VPD was only significant (decreasing duration) in well-watered plants.

Water regime, and to an extent VPD, affected the stable carbon isotope composition ($\delta^{13}C$) of plant parts sampled at mid-grain filling and maturity as well as that of mature grains (Table 4). Either full watering or low VPD during growth tended to decrease $\delta^{13}C$, the differences between treatments being maximal in mature grains.

Ψ_w decreased significantly in response to water deficit only at high VPD, but the RWC decreased under water stress in both VPD regimes (Table 5). There were significant VPD×soil water level interactions for both treatments. ALDM at mid-grain filling was greater in plants growing at high VPD and no effect of water status on this trait was observed. Chlorophyll content only decreased significantly as response to water stress when plants were grown at low VPD (Table 5).

P_N of flag leaves decreased significantly under water deficit at both VPD (Fig. 1). Both soil moisture and VPD had not effect on R_D of either the flag leaf or the ear. However, P_N of ears was higher at high VPD, and did not decrease under water stress. As a consequence, the carbon balance (as indicated by the P_N/R_D ratio) of leaf decreased considerably under water stress, whereas the ear P_N/R_D ratio decreased only at low VPD (Fig. 2). Combining all treatments together, grain yield was positively correlated with P_N of the ear (r^2 = 0.48, p <0.001) and also, but only to some extent, with that of the flag leaf (r^2 = 0.28, p <0.001).

Discussion

The effect of water regime and VPD on grain growth and yield: Plants growing at high VPD, either under well-watered or water deficit conditions, had higher grain yield and grain filling rate than plants growing at low VPD (Tables 1 and 3). The analysis of yield components revealed that low VPD significantly increased the number of (fully) sterile ears (Table 2). By contrast, water stress decreased grain yield and individual grain DM at any VPD. Nevertheless, the effect of water stress was higher under low VPD. Thus we found that at high VPD reduction in individual grain DM, as response to water stress, resulted primarily from decreases in duration of grain filling, whereas at low VPD also decreases in grain filling rate contributed significantly to lower grain yield of water stressed plants (Table 3). Water stress in barley reduces individual grain DM, particularly when combined with high temperature, due to reduction in duration of grain filling (Savin and Nicolas 1996). These authors concluded that combined water stress and high temperature strongly affected grain dry matter and yield. In cultivars of wheat most tolerant to high temperature during grain filling (*i.e.* showing the least reduction of grain dry matter at maturity) the rate of grain filling increased and was compensated for the reduced duration of grain filling (Wardlaw and Moncur 1995).

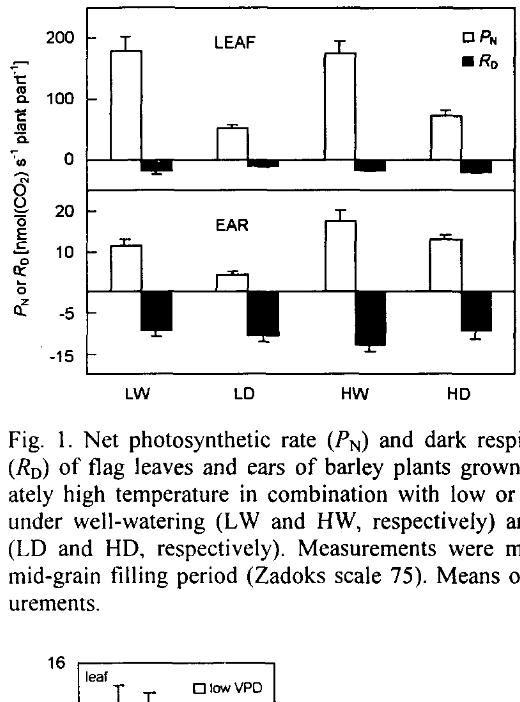

Fig. 1. Net photosynthetic rate (P_N) and dark respiration rate (R_D) of flag leaves and ears of barley plants grown at moderately high temperature in combination with low or high VPD under well-watering (LW and HW, respectively) and drought (LD and HD, respectively). Measurements were made at the mid-grain filling period (Zadoks scale 75). Means of 20 measurements.

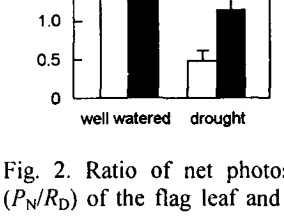

Fig. 2. Ratio of net photosynthetic rate to dark respiration (P_N/R_D) of the flag leaf and the ear of barley plants grown at moderately high temperature in combination with low or high VPD under well-watered and drought conditions. Otherwise as for Fig. 1.

Reduction of grain yield can also be attributed to aborted ears. Water stress increased the number of sterile ears (Table 2). Therefore, ear sterility increased in both VPD regimes in response to water stress, but was greater at low VPD. Grain abortion, due to exposure to a maximum temperature of 31 °C, was reported in wheat (Wheeler *et al.* 1996, Ferris *et al.* 1998). Grain abortion under high temperature depends, at least in part, on sink limitation (Ferris *et al.* 1998).

Effect of water regime and VPD on transpiration efficiency and $\delta^{13}C$: Apart from the soil water status, the key environmental factor influencing transpiration efficiency (TE) is the VPD during growth (Fischer and Turner 1978, Richards 1991, Condon *et al.* 1992). In our experiments TE calculated on grain yield basis (TE_y) was significantly affected by VPD, whereas water deficit, even though tending to increase TE_y, showed no significant effect. Thus, high VPD led to larger TE_y, either under well-watered or stressed conditions. By contrast, TE calculated on the basis of total plant dry matter at maturity (TE_p), increased in response to water deficit, with no effect associated with VPD (Table 1). For a given VPD the variation in water use efficiency of photosynthesis (WUE_{ph}), and in the long term also TE, arises from either differences in photosynthetic rate or stomatal conductance, or both. Under water deficit, stomata would close, limiting transpiration rate more than photosynthetic CO_2 uptake, which in turn will lead to an increase in WUE_{ph} (Larcher 1995). This agrees with the higher TE_p of barley plants under water deficit conditions. Regarding TE_y, the positive effect of a high VPD on this trait appears at first opposite to what would be expected when considered the purely physical role of VPD determining the amount of evaporative losses (Schulze 1986, Grantz 1990). However, under moderately high temperature, a high VPD had a positive effect on grain yield per plant, limiting the amount of sterile ears per plant (Table 2), which would lead to a larger TE_y. In addition, higher VPD seemed to act positively on the photosynthetic performance of leaves during grain filling. Thus the higher ALDM at mid-grain filling of plants growing at high VPD would be associated with a large amount of photosynthates currently produced by the flag leaf (Araus and Tapia 1987). Additionally, under water stress, chlorophyll content remained higher in plants growing at high VPD (Table 5). High temperature and water deficit strongly affected the specific leaf area (inverse of ALDM) in peanut (Craufurd *et al.* 1999). In addition, these authors also showed a negative correlation between WUE and specific leaf area. In our case, VPD was the only factor influencing ALDM, but in agreement with Craufurd *et al.* (1999) plants growing at high VPD exhibited both higher TE_y and ALDM.

For a given steady VPD, carbon isotope composition ($\delta^{13}C$), when measured as dry matter of C_3 plants, integrates the WUE_{ph} over the period during which the dry mass was laid down (Farquhar and Richards 1984, Condon *et al.* 1990). In this sense, a positive relationship between $\delta^{13}C$ and TE for a range of C_3 species (Condon *et al.* 1990, Ehleringer *et al.* 1991, Craufurd *et al.* 1999), including barley (Hubick and Farquhar 1989), was reported. Provided a steady VPD, the relationship between $\delta^{13}C$ and either WUE_{ph} or TE is just sustained through the negative relationship between $\delta^{13}C$ and the ratio of the internal *versus* the atmospheric partial pressures of CO_2 (p_i/p_a) of photosynthetic organs (Farquhar *et al.* 1989). In our study mature grains were the plant part having the largest differences in $\delta^{13}C$ between treatments (Table 4), which would agree with the role of grains as a sink for different photosynthetic plant organs. Water stressed plants showed higher (*i.e.* less negative) values of $\delta^{13}C$ suggesting that these plants exhibited higher TE than control plants (Table 4). The trend towards a higher $\delta^{13}C$ in response to water deficit was more evident when plants grew at high VPD. Indeed higher VPD increased $\delta^{13}C$, not only under water deficit but even (though to a lesser extent) in fully watered plants (Table 4). Because of the negative relationship between $\delta^{13}C$ and p_i/p_a, the plants growing under higher VPD would show systematically lower p_i. The lower internal CO_2 partial pressure in these plants would be due to the fact that stomata would remain more closed in response to the higher evaporative demand (*i.e.* higher VPD) of the surrounding air. Whereas the effect of a higher growth VPD is translated to a higher $\delta^{13}C$, this is not necessarily true when considering TE or the total amount of water transpired by the plant. Indeed, the effect of a higher VPD increasing the evaporative demand, and thus the transpiration rate for a given degree of stomata aperture, may compensate for its parallel effect on closing stomata. This would explain the lack of effect of a higher VPD on the total water transpired by plants, as well as its effect on increasing TE_p only in water stressed plants (Table 1).

Photosynthetic contribution of the ear and the flag leaf to grain yield: P_N/R_D ratios were much higher in leaves than in ears, due to proportionally higher R_D of the ears (Fig. 2). This fact is a consequence of respiration of grains and other non-photosynthesising tissues of the ears, which agrees with previous reports in durum wheat (Araus *et al.* 1993) and barley (Bort *et al.* 1994, 1996). P_N of flag leaves decreased significantly under water stress at both VPD regimes (Fig. 1). However, P_N of ears was higher at high VPD than at low VPD, and did not decrease in response to water stress. In durum wheat, most of the photosynthates in the grain come from ear parts and not from flag leaves (Araus *et al.* 1993). In barley, CO_2 exchange rates of ear correspond mainly to activity of awns (Blum 1985). The contribution of awns to gas exchange of the whole ear reached 90 % two weeks after anthesis (Ziegler-Jöns 1989). Indeed, under Mediterranean conditions photosynthesis by awns contributes more to barley yield than the leaf blades (Bort *et al.* 1994). The positive significant correlation between grain yield and P_N of ears, and its much lower correlation with P_N of the flag leaf, support the conclusion that maintenance of P_N in ears under water stress is an important factor determining the good performance (in terms of yield) of plants grown under water deficit and high VPD.

In summary, we found that the deleterious effects of combined moderately high temperature and water deficit on yield of barley was attenuated at high VPD. Plants subjected to these environments exhibited higher yield, TE_y, and $\delta^{13}C$. This may be related to the high capacity of the ear which is the main photosynthetic organ providing assimilates for filling the grain under drought.

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