



UNIVERSITAT DE BARCELONA



**FOTOSÍNTESIS, FOTOPROTECCIÓN, PRODUCTIVIDAD Y
ESTRÉS ABIÓTICO: ALGUNOS CASOS DE ESTUDIO**

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7. Does a higher yield potential improve barley performance in Mediterranean conditions?: a case study

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7.0. Abstract

Barley is one of the most widely cultivated crop in rainfed areas of the Mediterranean, where drought is the main factor that limits yield. Knowledge of the physiological traits responsible for adaptation of barley cultivars to Mediterranean environments may be relevant for future breeding strategies. Yield potential versus drought tolerance remains an open debate. Here we studied two barley cultivars (Graphic and Kym), of similar time to anthesis and crop duration, that are widely cultivated in the western Mediterranean. Grain yield was evaluated in 41 field trials and ranged (averaged for the 16-32 cultivars assayed in each trial) from 0.7 Mg ha⁻¹ to 9.1 Mg ha⁻¹. Yield components and carbon isotope discrimination ($\Delta^{13}\text{C}$) of grains was analysed in another two trials. Graphic production was almost 20% greater than Kym in all conditions. This greater yield was sustained mainly by more ears per unit ground area, and secondarily by the larger number of kernels per spike, which indicated that Graphic has a higher growth potential than Kym during tillering, the first part of the crop cycle. Moreover, Graphic showed greater $\Delta^{13}\text{C}$ of kernels, indicating improved water status even at the end of the crop cycle. To examine differences in early-growth, these cultivars were grown in optimal conditions and then photosynthetic activity and biomass analysed at the end of tillering. Graphic showed greater above-ground and root biomass as well as total leaf area per plant and per tiller than Kym, and also tended to have more tillers per plant, but its shoot-to-root biomass ratio was lower. Nitrogen content per unit leaf area was correlated negatively with plant and with tiller leaf area and positively with the shoot-to-root biomass ratio. Photosynthetic rate per unit leaf area was lower in Graphic and positively related to a lower nitrogen content, whereas stomatal limitation of photosynthesis and water use efficiency was similar in the two cultivars. Ribulose 1,5-biphosphate regeneration capacity contributed to the lower photosynthetic rate of Graphic. Moreover, quantum yield of PSII electron transport was also lower in Graphic than Kym, which suggests that mechanisms other than leaf structure also contributed to the higher photosynthetic capacity of the former. Nevertheless, as result of differences in leaf area, total plant photosynthesis was greater in Graphic.

We concluded that the higher yield of Graphic under a wide range of Mediterranean conditions is sustained by increased plant growth and total photosynthesis during tillering, even when the photosynthetic capacity per unit area of leaves is lower than that of Kym. Graphic has a more extensive root system than Kym, subsequently improving its water

status in later stages of the crop cycle. Nitrogen content per unit leaf area is a good indicator of the growth and photosynthetic activity of barley plants in the early stages of the crop cycle.

Abbreviations used: A , net CO₂ assimilation rate; A_{max} , CO₂-saturated net CO₂ assimilation rate; A_{sat} , light-saturated net CO₂ assimilation rate; c_a , c_i ambient and intercellular CO₂ concentration respectively; DW , dry weight; $\Delta^{13}C$, carbon isotope discrimination; F_m and F_m' , maximum fluorescence in dark-adapted and light-adapted leaves respectively; F_v/F_m , maximum efficiency of *PSII* photochemistry after dark-adaptation; F_v'/F_m' , efficiency of energy capture by open *PSII* centers; F_o' , minimum fluorescence yield in light-adapted state; ϕ_{PSII} , quantum yield of *PSII* electron transport; g_s , stomatal conductance; $J_{max,RuBP}$, maximum potential rate of electron transport contributing to ribulose 1,5-biphosphate regeneration; $J_{max,PSII}$, the rate of *PSII* electron transport in saturating light and CO₂; l , stomatal limitation to A_{sat} ; PPFD, photosynthetic active photon flux density; *PSII*, photosystem II; qP , photochemical quenching of chlorophyll fluorescence; R_d , dark respiration; RuBP, ribulose 1,5-bisphosphate; RWC, relative leaf water content; TR , transpiration rate; $V_{c,max}$, maximum carboxylation velocity of Rubisco; WUE , water use efficiency; ψ_w , leaf water potential.

7.1. Introduction

Plant growth and yield are severely reduced by water deficit, drought being the main abiotic stress factor that limits the production of cereals and other major crops in Mediterranean conditions (Acevedo et al. 1999; Araus 2002). Barley is one of the most widely cultivated cereals in the Mediterranean region, where it is grown even in the driest areas.

Knowledge of the traits responsible for adaptation of barley to Mediterranean environments may be relevant for future breeding strategies. In Mediterranean rainfed conditions, genetic improvement through breeding is frequently hindered by a large interaction between genotype and environment (either season or location), which mainly arise from unpredictable rainfall (Richards et al. 2002). Thus, when grown under harsh environments the genotypes selected for poor environmental conditions probably perform better than those released for high-yielding environments and *vice-versa*. From an ecophysiological perspective, drought responses may be initially divided into two categories: those that confer tolerance to extreme stress, and those that maximize productivity under less extreme conditions. Drought tolerance may incur penalties in yield under less extreme conditions, while traits maximizing productivity are fully expressed in the absence of stress (i.e. constitutive traits) although they still sustain yields under mild to moderate drought (Blum 1996; Araus et al. 2002a). Thus for growing areas other than the very drought-prone environments proposed barley “ideotypes” should have minimal genotype by environment interaction, showing both high yield potential and yield stability. Hence selection for greater yield potential has frequently resulted in higher production in a wide range of environments (Slafer et al. 1999; Richards 2000; Richards et al. 2002; Araus et al. 2002a). However, for drought-prone environments with barley yields often below 1.0 Mg ha⁻¹, breeding efforts to improve survival (i.e. tolerance to severe stress) and thus yield stability have been successful (Ceccarelli and Grando 1996). In these conditions locally adapted germplasm has been used (Ceccarelli et al. 1998). Therefore, there is a general agreement that for barley a high yield potential is advantageous under moderate stress conditions, whereas advantages from drought tolerance of a cultivar with low yield potential may be expressed only when stress is severe (Voltas et al. 1999).

A fast growth before anthesis has been reported as very important in Mediterranean type climate, where a terminal drought episode during the spring is probable (López-Castañeda *et al.* 1995 and references cited therein). In fact, this trait seem to be the reason for

the success of barley compared with other cereals in these environments. The advantage of early growth will arise from a higher water use efficiency because growth take place when it is cool (winter) and a reduction in water lost from soil surface (e.g. Blum 1996). In this sense, although it has been considered that high yield potential and drought tolerance are mutually exclusive (e.g. Blum 1996), a higher biomass accumulation before anthesis could be compatible to both traits (López-Castañeda et al. 1995).

At first sight, plant growth is influenced by the amount of photosynthetically active radiation intercepted by the plant (which is related to leaf area) and the efficiency with radiation (*RUE*, i.e. photosynthetic rate / *PPFD*) is used (Smith et al. 1999). Although the implication of photosynthetic rate in final grain yield is largely debatable (e.g. Reynolds et al. 2000), it could be useful its analysis to understand growth characteristics, in particular at early stages of the crops. Because photosynthetic rate depend on stomatal and non-stomatal (i.e. metabolic) factors, we discriminated both type of effects mediating the photosynthesis / CO₂ response (*A/C_i* curves) in simultaneous with modulated chlorophyll fluorescence (e.g. Nogués and Baker 2000).

Here we analyzed two barley genotypes with a similar crop cycle, Graphic and Kym, which have been extensively cultivated in the Western Mediterranean basin over the last decade, and which are currently used as control varieties in official Spanish trials for registration purposes. Both are British cultivars bred for high yielding conditions and released in 1978 (Kym) and 1990 (Graphic).

The present article include three experimental approach. First, we analyze the yield performance of both cultivars throughout an extensive environmental range, from highly favourable to very harsh conditions. Second, in order to explore the causes of different yield performance, two field trials were carried out. In this experiments, we measured the components of grain yield and carbon isotopic discrimination (as an estimation of water use efficiency). Finally, we performed an experiment in semi-controlled conditions (greenhouse), analyzing plant characteristics (e.g. tillering, biomass partition, leaf area) and the physiological factors (in particular photosynthetic parameters) underlying the different performance of both cultivars at early growth.

7.2. Material and Methods

7.2.1. Field studies

7.2.1.1. Yield and its agronomic components

Two spring two-rowed barley (*Hordeum vulgare* L.) cultivars (Graphic and Kym) were studied. Yield was recorded from a set of 41 field trials of the Spanish Registration Office for Varieties (Oficina Española de Variedades Vegetales) performed in 1999, 2000 and 2001 in several locations throughout Spain which represent a wide range of environmental conditions (Fig. 1). In each case, conventional farming techniques, and a sowing density of 350 seeds m⁻² were used. The location and climatic conditions of the trials and agronomic details are described elsewhere (O.E.V.V. 2001). The experimental set-up for each trial was a randomized complete block design with four replications. Plants were harvested at maturity and yield was then calculated

In addition to the above trials, two randomised, complete block field trials with three replications were done in the moderate to harsh semi-arid Mediterranean environments of north-eastern Spain (Lleida province). Plots consisted of six 12-m rows, spaced 20 cm apart, seeded at rates of 350 seeds m⁻², and fertilised following standard agricultural practices. At maturity, grain yield and agronomic components of yield (ears m⁻², kernels per ear and individual grain weight) and harvest index (*HI*, the ratio of grain yield to total above-ground biomass) were calculated. As no significant interaction was observed between trials for any of the traits studied, data were pooled for statistical analysis (Table 1).

7.2.1.2. Carbon isotope discrimination

Kernels from the two trials in Lleida were oven-dried and ground to a fine powder. ¹³C/¹²C ratios were calculated by mass spectrometry at the Isotope Services (Los Alamos, New Mexico, US). Results were expressed as δ¹³C values where:

$$\delta^{13}\text{C} (\text{‰}) = \left[\left(\frac{R_{\text{sample}}}{R_{\text{standard}}} \right) - 1 \right] \times 1000 \quad (1)$$

with R is the ¹³C / ¹²C ratio, using a secondary standard calibrated against Pee Dee Belemnite (PDB) carbonate. Accuracy of the δ¹³C measurements was greater than 0.1‰. Carbon isotope discrimination (Δ) in grains was calculated from δ_a and δ_p, as:

$$\Delta^{13}\text{C} = (\delta_a - \delta_p) / (\delta_p + 1) \quad (2)$$

where a and p refer to air and plant, respectively. The value of $\delta^{13}\text{C}$ for the air was assumed to be -8.00‰ (Farquhar et al. 1989).

7.2.2. Early crop growth

7.2.2.1. Experimental set-up

The two cultivars were grown in pots for eight weeks in a ventilated greenhouse at the Experimental Fields of the University of Barcelona in a fully randomised factorial experiment. At the time of measuring and sampling, plants had attained stage 29 according to Zadoks' decimal code (Zadoks et al. 1974) which corresponds to the end of tillering. Plants were grown in containers of 1-L filled with peat:perlite:vermiculite (2:1:1 v/v) and fertilized daily with Hoagland's solution (Hoagland 1937). For each cultivar, five pots, each containing one plant, were grown under mean day/night temperatures and maximum photosynthetic photon flux density (*PPFD*) of about 25 °C/15°C and about 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$, respectively. To avoid water evaporation from substrate, the tops of the pots were sealed with cellulose film. The youngest fully expanded leaves of the main stem were used for all measurements.

7.2.2.2. Gas exchange measurements

To examine photosynthetic activity and stomatal and non-stomatal limitations of photosynthesis, gas exchange and chlorophyll fluorescence measurements were taken in the last fully-expanded leaf at the end of tillering (Nogués et al. 1998). Gas exchange was measured between 9:00 a.m. and 7:00 p.m. using an infrared gas analysis (*IRGA*) system (LI-COR 6400, LI-COR Inc., Lincoln, NB, USA). At least one leaf per pot was measured. Plants were supplemented with 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ of *PPFD* for at least 30 min before measurements were taken, and no effect on stomatal conductance caused by the daily pattern was observed during measurements. The leaf chamber was maintained at 25°C with a saturating *PPFD* of 1200 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Net CO_2 assimilation rate (*A*) and intercellular CO_2 (*C_i*) concentration were calculated following von Caemmerer and Farquhar (1981). Light-saturated net CO_2 assimilation rate (*A_{sat}*) was measured at the above *PPFD* and at an ambient CO_2 (*C_a*) concentration of 360 $\mu\text{mol mol}^{-1}$. In addition to the CO_2 -saturated net

CO₂ assimilation rate (A_{max}), several photosynthetic parameters were derived from the A/C_i curves. Stomatal limitation (I), which is the proportional decrease in light-saturated A attributable to stomata, was calculated as $[(A_0 - A_{sat})/A_0] \times 100$, where A_0 is A at C_i of 360 $\mu\text{mol mol}^{-1}$ (Farquhar and Sharkey 1982). Estimation of maximum carboxylation velocity of Rubisco ($V_{c,max}$) and maximum potential rate of electron transport ($J_{max,RuBP}$) contributing to ribulose 1,5-biphosphate regeneration were made by fitting a maximum likelihood regression below and above the inflexion of A/C_i response, as reported by McMurtrie and Wang (1993). Dark respiration (R_d) was measured after 10 min of darkness in the same leaves used for the A/C_i curves.

7.2.2.3. Chlorophyll fluorescence measurements

Steady-state chlorophyll fluorescence of leaves was measured using a modulated chlorophyll fluorimeter (MiniPAM, Walz, Effeltrich, Germany) simultaneously with gas exchange measurements. The leaf chamber of the IRGA was modified to accept the fiber optic from the modulated fluorimeter (Nogués and Alegre 2002). Fluorescence was analysed (Andrews et al., 1993) to estimate the relative quantum yield of PSII [$\phi_{PSII} = (F_m' - F_s) / F_m'$], efficiency of energy capture by open PSII centres [$F_v'/F_m' = (F_m' - F_o') / F_m'$] and photochemical quenching [$qP = (F_m' - F_s) / (F_m - F_o')$]. The potential quantum yield of PSII was calculated in leaves after 15 minutes of dark adaptation (Nogués et al. 1998). Minimum fluorescence yield in the light-adapted state (F_o') was estimated following Oxborough and Baker (1997). The rate of PSII electron transport at saturating light and CO₂ ($J_{max,PSII}$) was estimated as described by Allen et al. (1997).

7.2.2.4. Water status

Water potential (ψ_w) and relative water content (RWC) were measured in leaves similar to those used for gas exchange and fluorescence measurements. The former parameter was taken using a pressure chamber (ARIMAD-2, ARI Far Charuv-Water Supply Accessories, Israel) closed with damp paper to decrease evaporation. For each RWC measurement, a leaf section (30 mm length) was weighed (w_i), floated on distilled water at 4°C overnight, reweighed (w_f), and then dried at 80°C for 48 h, after which dry weight (w_d) was determined. RWC was calculated as:

$$RWC = (w_i - w_d) (w_f - w_d)^{-1} \times 100$$

7.2.2.5. Biomass analysis

At the end of the photosynthetic measurements, plants were harvested, and height was measured from the bottom to the tip of the topmost leaf of the plant. Leaves were scanned and total area was estimated with an image-processing program (Nogués and Alegre 2002). Plants were then dried at 80°C, and the biomass of shoots and roots was measured. Specific leaf area was calculated as the ratio of leaf area to *DW*.

7.2.2.6. Carbon isotope discrimination and nitrogen analysis

Samples of the leaves used for the photosynthetic measurements were analysed for stable carbon isotope and nitrogen content. About 10 mg of dry matter was finely ground for each leaf blade. Total nitrogen content of leaves was measured on 0.7-0.9 mg samples burnt combusted in an elemental analyser (EA1108, Series 1, Carlo Erba Instrumentazione, Milan, Italy). The $^{13}\text{C}/^{12}\text{C}$ ratio was then measured on an isotope ratio mass spectrometer (Delta C, Finnigan Mat, Bremen, Germany) operated in continuous flow mode. Measurements were done at the Scientific and Technical Services of the University of Barcelona, Spain. A system check for elemental analyses was performed with an interspersed working standard of atropine (Carlo Erba, Milan, Italy). Stable carbon isotope composition was expressed as $\delta^{13}\text{C}$ values, following Farquhar et al. (1989) using secondary standards of graphite, sucrose and polyethylene foil (IAEA, Vienna, Austria) calibrated against PDB carbonate. The accuracy of the $\delta^{13}\text{C}$ measurements was $\pm 0.1\%$. $\Delta^{13}\text{C}$ was then also calculated as above.

7.2.2.7. Statistical analyses

Linear regressions and non-linear curves were fitted using SigmaPlot v8.0 (SPSS Inc.). Heterogeneity of slopes ANOVA and LSD tests were carried out using standard SAS-STAT procedures (Little et al. 1991). Unless otherwise stated, differences were considered statistically significant when $P < 0.05$.

7.3. Results and Discussion

7.3.1. Yield performance

We compared the performance of the two barley cultivars along a set of 41 field trials using the model described by Finley and Wilkinson (1963). This model graphically illustrates the comparative performance of distinct genotypes in a range of environmental conditions and shows the yield response to the environmental index, described as the average yield of all participating genotypes. In our experiment the slope of the relationship between grain yield and the environmental index (Fig. 1) was significantly higher for Graphic than Kym, but there was no crossover and no significant differences between lines. Thus, from the fitting equations (Fig. 1) Graphic yielded from 16.3% to 35.0% more than Kym in highly favourable environmental conditions (with an environmental index of 9.1 Mg ha⁻¹) and in drought conditions (with 0.7 Mg ha⁻¹), respectively.

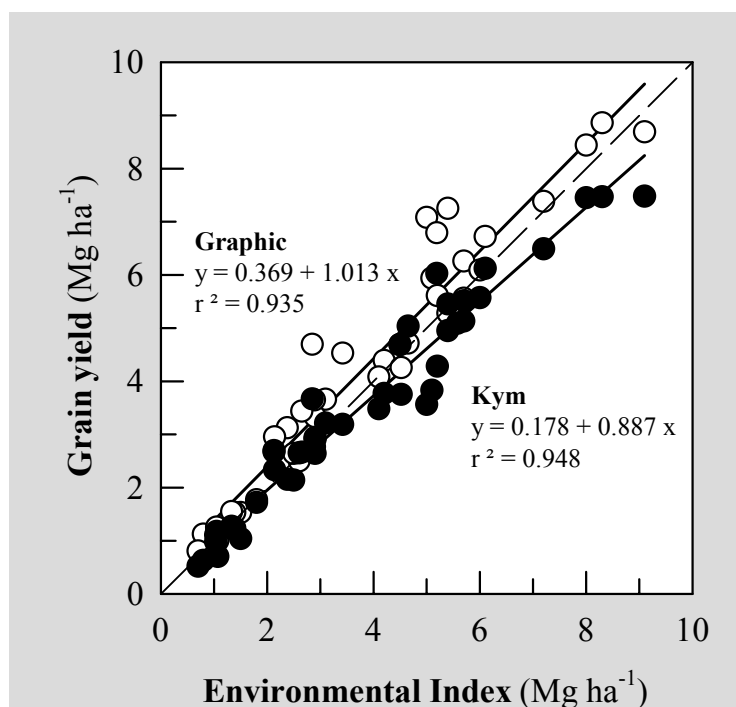


Figure 1. The relationship between grain yield and the Environmental Index for the barley cultivars Graphic (○) and Kym (●). This Index was calculated as the average grain yield of all the cultivars assayed in each location (from 16 to 32) of the 41 field trials of the Spanish registration office. Each point represents the mean of four replicates (blocks) in each location. Linear regressions and determination coefficients (r^2) are shown for each cultivar.

In the model proposed by Finley and Wilkinson (1963), if the range of the environmental (E) index and genotype (G) variation is large enough, a negative $G \times E$ interaction is frequently observed, as shown by a crossover in the yield of the genotypes of high and low yield potential. Thus, when grown under very poor environments, the cultivars selected for low yield conditions probably perform better than those released for high-yielding environments and *vice-versa*. Normally, this crossover is placed at low yields, but appears to be slightly higher for barley (2.0-2.5 Mg ha⁻¹; Ceccarelli et al. 1991;

Volta et al. 1999) than for wheat (1.0-1.5 Mg ha⁻¹; Laing and Fischer 1977; Fischer 1993). However in our study, Graphic performed better than Kym in optimal conditions and, to a somewhat lesser extent, in drought-prone environments, suggesting that differences between the two barley cultivars across a wide range of environmental conditions are explained, at least in part, by a higher yield potential in the former. In other words, despite that Graphic seem to be less stable than Kym (compare the slopes in regression plots in Fig. 1), crossover is not observed. Reports on wheat show that modern cultivars outyield their older counterparts even in the lowest-yielding conditions (Slafer and Andrade 1993; Calderini et al. 1995; Perry and D'Antuono 1989). In our results, the lack of crossover even in the poorest environment assayed (Fig. 1) may be due to the fact that both genotypes were selected for high yielding conditions.

Analysis of the agronomic components of grain yield, in the two trials where these traits were studied, showed that ear number per unit grown area and the number of kernels per ear were the main traits determining differences in yield between cultivars. However, grain weight and harvest index did not differ consistently between cultivars (Table 1). The number of ears is determined by tillering capacity and further during stem elongation by the rate of tiller survival, whereas the photosynthetic area of the tiller determines the number of spikelets per spike (Slafer et al. 1994; Smith et al. 1999). Although the relationship between tiller number and ear m⁻² can be taken with caution (e.g. changes in ear m⁻² can occur independent of tillering; see references in Smith et al. 1999) differences between genotypes in early phases of the crop cycle may be the primary cause of differences in yield.

Kernel $\Delta^{13}\text{C}$ of Graphic was higher than that of Kym in both trials (Table 1). Analysis of $\Delta^{13}\text{C}$ is a reliable method to evaluate water use efficiency (*WUE*) in barley (Hubick and Farquhar 1989; Acevedo 1993). The higher $\Delta^{13}\text{C}$ observed in Graphic in both trials (Table 1) indicates a larger ratio of intercellular to ambient CO₂ concentration (c_i/c_a), which is probably due to higher stomatal conductance and thus (providing there are no differences in vapour pressure deficit) a lower *WUE*. However, $\Delta^{13}\text{C}$ depends, indirectly, on the water available during growth (Araus *et al.* 1997) and ultimately on the water transpired (Araus et al. 2002b). Thus a positive relationship across genotypes between $\Delta^{13}\text{C}$ and yield has been widely reported in C₃ cereals grown in mild to moderate drought conditions (Richards et al. 2002; Araus et al. 2002a, 2003). When the date of anthesis does not differ between strains, as in the present study (Table 1), this relationship may imply

that genotypes with a higher $\Delta^{13}\text{C}$ have used more water (absorbed and transpired) under drought. Therefore the higher kernel $\Delta^{13}\text{C}$ of Graphic indicates a better water status than Kym. As the greater $\Delta^{13}\text{C}$ of Graphic was not related with an earlier anthesis date, other factors such as greater root development could be responsible for its better water status (Blum 1996).

In summary, differences in yield between Graphic and Kym in the absence of stress seem to be established during tillering, when the first agronomic yield components of the crop, number of ears per unit ground area and (at least in part) number of kernels per spike, are determined. Furthermore, $\Delta^{13}\text{C}$ values indicate a better water status (more water transpired) of Graphic than Kym. Allocating evaporation into transpiration by increasing early vigour (the capacity for fast leaf-growth) or capturing more water for transpiration, through increased root growth, are options suggested to improve yield of wheat under water stress (Araus 2002; Richards et al. 2002).

7.3.2. Performance during tillering

To better enhance genotype differences in growth pattern, while avoiding the potential interaction of low temperature, which is common during tillering, plants were grown from sowing to the end of tillering in a greenhouse under optimal water and temperature conditions. Moreover in Mediterranean conditions growth during the first part of the crop cycle in the cereals usually occurs in well-watered conditions, with ratios of rainfall to evapotranspiration frequently higher than one (Acevedo et al. 1999). Thus in our study at the end of the growing period the two genotypes showed similar water potential (ψ_w , *ca.* -0.6 MPa) and relative water content (95.0%) (Table 2). Moreover, intercellular CO_2 concentrations (c_i) were also similar and over 70% of c_a , a typical percentage for non-stressed C_3 species (Drake et al. 1997). Accordingly, very high $\Delta^{13}\text{C}$ values (*ca.* 24.5‰) were observed in Graphic and Kym, which were in the range reported for barley seedlings grown in the field under well watered conditions (Craufurd et al. 1991; Acevedo 1993).

Table 1.- Mean grain yield, agronomic yield components and carbon isotope discrimination ($\Delta^{13}\text{C}$) of two field trials of barley (*Hordeum vulgare* L.) cvs. Graphic and Kym carried out in Lleida (NE Spain) in rainfed conditions. TGW : thousand grain weight.

Trial	Genotype	Yield (Mg ha ⁻¹)	ears m⁻²	kernels ear⁻¹	TGW (g)	Harvest Index	$\Delta^{13}\text{C}$ grain (‰)	Anthesis Date
I	GRAPHIC	3.64 ± 0.40	466 ± 32.2	24.8 ± 0.70	30.8 ± 1.55	0.43 ± 0.004	16.78 ± 0.20	3 May
	KYM	2.76 ± 0.14	388 ± 48.5	24.0 ± 0.53	30.1 ± 4.25	0.42 ± 0.046	16.06 ± 0.21	1 May
II	GRAPHIC	4.53 ± 0.51	568 ± 54.2	25.5 ± 0.37	40.3 ± 0.62	0.49 ± 0.018	16.69 ± 0.24	6 May
	KYM	3.19 ± 0.28	430 ± 65.3	24.2 ± 0.23	33.8 ± 0.55	0.39 ± 0.032	15.95 ± 0.08	8 May

7.3.2.1. Plant growth

Leaf number per plant and individual leaf area were 29% and 19% higher, respectively, in Graphic than in Kym. Consequently, the total leaf area of Graphic per plant and per tiller was 61 and 33%, respectively, greater in Graphic than in Kym (Table 2). Larger tillers may improve survival (Slafer et al. 1994, 1999; Miralles and Slafer 1999) and then Graphic showed more reproductive tillers during stem elongation as well as larger spikes with more spikelets than Kym. In addition, a greater number of leaves per plant implies more tillers, and eventually (but not always, see section *Yield performance*) more ears per plant (Slafer et al. 1994, 1999). In our study mean value of tillers per plant was 20% higher in Graphic than Kym, even when differences were only significant at a higher level of probability ($P = 0.13$). Specific leaf area (*SLA*, the ratio of leaf area to *DW*) did not differ significantly ($P < 0.05$) between genotypes. However, leaf nitrogen content was lower in Graphic both per unit *DW* (17%) and per unit area (25%) (Table 2). Leaf nitrogen content per unit area was negatively correlated with total plant leaf area (Fig. 2A), and with total leaf area per tiller ($R^2 = 0.81$, $y = 91.60 + 32076 e^{(-7.31 * x)}$), but not with number of tillers per plant.

Moreover, Graphic had a higher total shoot biomass (40%) and leaf dry weight (37%) and a much larger increase (78%) in root *DW*. Therefore, the shoot-to-root biomass ratio was 32% lower than that of Kym. Given that the growing conditions were optimal, differences in this ratio between cultivars may be considered constitutive. Leaf nitrogen content over 1.3 gN m⁻² was associated with a strong increase in the shoot-to-root biomass ratio (Fig. 2B). Although the extrapolation of greenhouse results to the field must be made with caution, we cannot discard that a larger root development of Graphic could enhance water extraction in drought conditions in the field (Blum 1996). The lack of differences between cultivars in water status does not support mechanisms such as the action of abscisic acid action (Passioura 2002) or osmolyte accumulation (Serraj and Sinclair 2002).

Total crop biomass depends, among other factors, on the photosynthetic radiation intercepted by the canopy, which in turn depends on the total leaf area (Richards 2000). Assuming no change in other components, an increase in biomass leads to a higher potential grain yield (Hay 1999). Factors such as lower *SLA* and leaf nitrogen content per area may be related to an increase in leaf area (Porter 1990). The greater growth of barley than wheat during tillering has been partly attributed to a higher *SLA* in the former (López Castañeda et al.

1995, 1996). We did not observe significant differences in *SLA* between the two barley cultivars, but leaf nitrogen content was lower in Graphic (Table 2). Thus, a higher leaf area, related to a lower nitrogen investment, could lead to an increased interception of radiation and thus to greater biomass even assuming a reduction in radiation use efficiency. For instance, antisense Rubisco transformants in tobacco exhibited lower light-saturated net CO₂ assimilation rates (*A_{sat}*) but invested assimilates in leaf area development more efficiently (Reynolds et al. 2000).

Table 2. Plant growth characteristics of barley (*Hordeum vulgare* L.) cvs. Graphic and Kym at the end of tillering. Plants were grown for 8 weeks in optimal temperature and water conditions in a greenhouse prior to measurements and harvesting. DW, dry weight. Specific leaf area and nitrogen content values refer to the recently expanded leaf where gas exchange measurements were performed. The ratio GLA/GLN correspond to the plant-averaged-area of one leaf and the ratio GLA/LDW to the specific leaf area of the whole plant. Values are the mean \pm SE of four to five pots, each pot contained one plant. Means followed by the same letter are not significantly different at $P = 0.05$ according LSD test.

	GRAPHIC	KYM
Plant height (cm)	71.0 \pm 2.7 ^a	71.1 \pm 2.5 ^a
Total leaf number	50.8 \pm 4 ^a	39.5 \pm 5.4 ^b
Green leaf number per plant (GLN)	49.2 \pm 3.2 ^a	36.5 \pm 3.7 ^b
Green leaf area per plant (GLA) (cm²)	1551 \pm 112 ^a	962 \pm 94 ^b
GLA / GLN (cm²)	31.6 \pm 1.7 ^a	26.5 \pm 1.1 ^b
Number of tillers	12.25 \pm 0.25 ^a	10.25 \pm 1.11 ^a
Green leaf area per tiller (cm²)	127.1 \pm 19.8 ^a	95.4 \pm 16.1 ^b
Total Plant DW (g)	8.90 \pm 0.8 ^a	6.40 \pm 1.1 ^b
Leaf DW (LDW) (g)	3.80 \pm 0.4 ^a	2.40 \pm 0.3 ^b
Stem DW (g)	3.60 \pm 0.3 ^a	3.20 \pm 0.5 ^a
Root DW (g)	1.60 \pm 0.1 ^a	0.90 \pm 0.3 ^b
Shoot DW /root DW ratio	4.60 \pm 0.2 ^a	6.80 \pm 1.1 ^b
GLA/LDW (cm² g⁻¹ DW)	415.8 \pm 22.7 ^a	413.5 \pm 22.1 ^a
Specific leaf area (cm² g⁻¹ DW)	387.7 \pm 53 ^a	353.1 \pm 28 ^a
Leaf nitrogen content (% DW)	3.70 \pm 0.25 ^a	4.44 \pm 0.22 ^b
Leaf nitrogen content (g N m⁻²)	0.95 \pm 0.06 ^a	1.26 \pm 0.06 ^b

7.3.2.2. Photosynthetic activity

When net CO₂ assimilation rate (*A*) was expressed on leaf area the curves *A/c_i* clearly illustrated the differences in photosynthetic activity between the cultivars (Fig. 3A). *A_{sat}* was 22% higher in Kym than Graphic (Table 3), CO₂-saturated net CO₂ assimilation rate (*A_{max}*) and maximum potential rate of electron transport contributing to ribulose 1,5-biphosphate

regeneration ($J_{max,RuBP}$) on area basis were also higher (16% and 20% respectively) in Kym. However, when expressed on the basis of nitrogen content no differences were detected between cultivars for A_{sat} , A_{max} (Table 3) or for the other photosynthetic traits derived from the A/c_i curves (data not shown). In addition, maximum carboxylation velocity of Rubisco ($V_{c,max}$) and dark respiration did not differ significantly between genotypes, even when they tended also to be lower in Graphic than Kym. Leaf nitrogen content calculated on area basis was positively correlated ($P < 0.05$) with the photosynthetic parameters A_{sat} (Fig. 2C), and A_{max} , $J_{max,RuBP}$ and $V_{c,max}$, also expressed on area basis. These relationships clearly increased after removing one outlier value of Kym (e.g. $r^2 = 0.74$, for the relationship with A_{sat}). The positive correlation between leaf nitrogen content and photosynthetic rate on area basis reported in several species (Araus and Tapia 1987; Pons et al. 1990; Hay 1999; Evans and Porter 2001) could explain the differences in assimilation rate between Graphic and Kym (Table 2, Fig. 2C).

However, although Graphic showed a lower photosynthetic rate per unit leaf area, the rate of plant photosynthesis (calculated as the product of total green leaf area per A_{sat}) was 32% higher in Graphic than Kym. As reported for wheat (Austin 1999; Reynolds et al. 2000), breeding for a higher photosynthetic rate on an area basis could be irrelevant or even have negative consequences for yield. In fact, a negative relationship between leaf area and photosynthetic rate has been reported for wheat (Austin 1999). This negative correlation has been attributed to an increase in leaf thickness and/or tighter packing of mesophyll cells (even without an increase in leaf thickness) with an increased nitrogen content and a higher photosynthetic capacity in smaller leaves (Araus et al. 1986). In our study, even when no significant differences between cultivars were found in *SLA* (a simple indicator of the amount of photosynthetic tissue in leaves; Dijkstra 1990), differences in nitrogen content were nevertheless present. Therefore, differences in photosynthetic rate seem to be due to the packing of mesophyll cells rather than to leaf thickness.

Table 3.- Photosynthetic parameters derived from the A/c_i curves and modulated chlorophyll fluorescence, plus gas exchange parameters determining water use efficiency and carbon isotope discrimination, of barley (*Hordeum vulgare* L.) cvs. Graphic and Kym at the end of tillering. Plants were grown for 8 weeks in optimal temperature and water conditions in a greenhouse. Gas exchange and chlorophyll fluorescence was measured simultaneously in the same recently expanded leaves at $1200 \mu\text{mol m}^{-2} \text{s}^{-1}$ of PPFD and 25°C . Values presented for g_s , c_i and transpiration correspond to those obtained at the time of A_{sat} measurement. Fluorescence parameters were measured simultaneously with A_{sat} and except for $J_{\text{max,PSII}}$ are expressed in relative units. Values are the mean \pm SE of five replicates, each from separate pots. Means followed by the same letter are not significantly different at $P = 0.05$ according to an F-protected LSD test.

	GRAPHIC	KYM
Leaf water status		
ψ_w (- MPa)	0.69 ± 0.06^a	0.55 ± 0.05^a
RWC (%)	95.1 ± 0.9^a	94.9 ± 0.9^a
A / c_i parameters		
A_{sat} ($\mu\text{mol m}^{-2}\text{s}^{-1}$)	16.2 ± 0.9^a	19.7 ± 1.3^b
A_{sat} ($\mu\text{mol g N}^{-1}\text{s}^{-1}$)	17.0 ± 0.8^a	15.8 ± 1.3^a
I (%)	13.6 ± 1.3^a	11.8 ± 1.6^a
$V_{c, \text{max}}$ ($\mu\text{mol m}^{-2}\text{s}^{-1}$)	72.0 ± 4.2^a	86.9 ± 4.4^a
$J_{\text{max,RuBP}}$ ($\mu\text{mol m}^{-2}\text{s}^{-1}$)	143.2 ± 7.9^a	171.4 ± 10.7^b
A_{max} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	23.2 ± 1.2^a	27.0 ± 1.4^b
A_{max} ($\mu\text{mol g N}^{-1}\text{s}^{-1}$)	24.5 ± 1.1^a	21.6 ± 1.5^a
Modulated chlorophyll fluorescence		
Φ_{PSII}	0.326 ± 0.027^a	0.413 ± 0.017^b
F_v' / F_m'	0.573 ± 0.015^a	0.611 ± 0.011^a
qP	0.566 ± 0.033^a	0.674 ± 0.018^b
F_v / F_m	0.780 ± 0.009^a	0.798 ± 0.003^a
$J_{\text{max,PSII}}$ ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	183.4 ± 12.4^a	212.5 ± 8.6^a
Water use efficiency		
g_s ($\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$)	0.417 ± 0.048^a	0.500 ± 0.06^a
TR ($\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$)	3.96 ± 0.31^a	5.12 ± 0.60^b
WUE ($\mu\text{mol CO}_2 \text{mmol H}_2\text{O}^{-1}$)	4.15 ± 0.28^a	4.01 ± 0.45^a
Rd ($\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$)	0.787 ± 0.21^a	0.963 ± 0.20^a
c_i ($c_a = 360 \mu\text{mol mol}^{-1}$)	280.8 ± 6.0^a	276.8 ± 5.4^a
$\Delta^{13}\text{C}$ (‰)	24.6 ± 0.3^a	24.4 ± 0.2^a

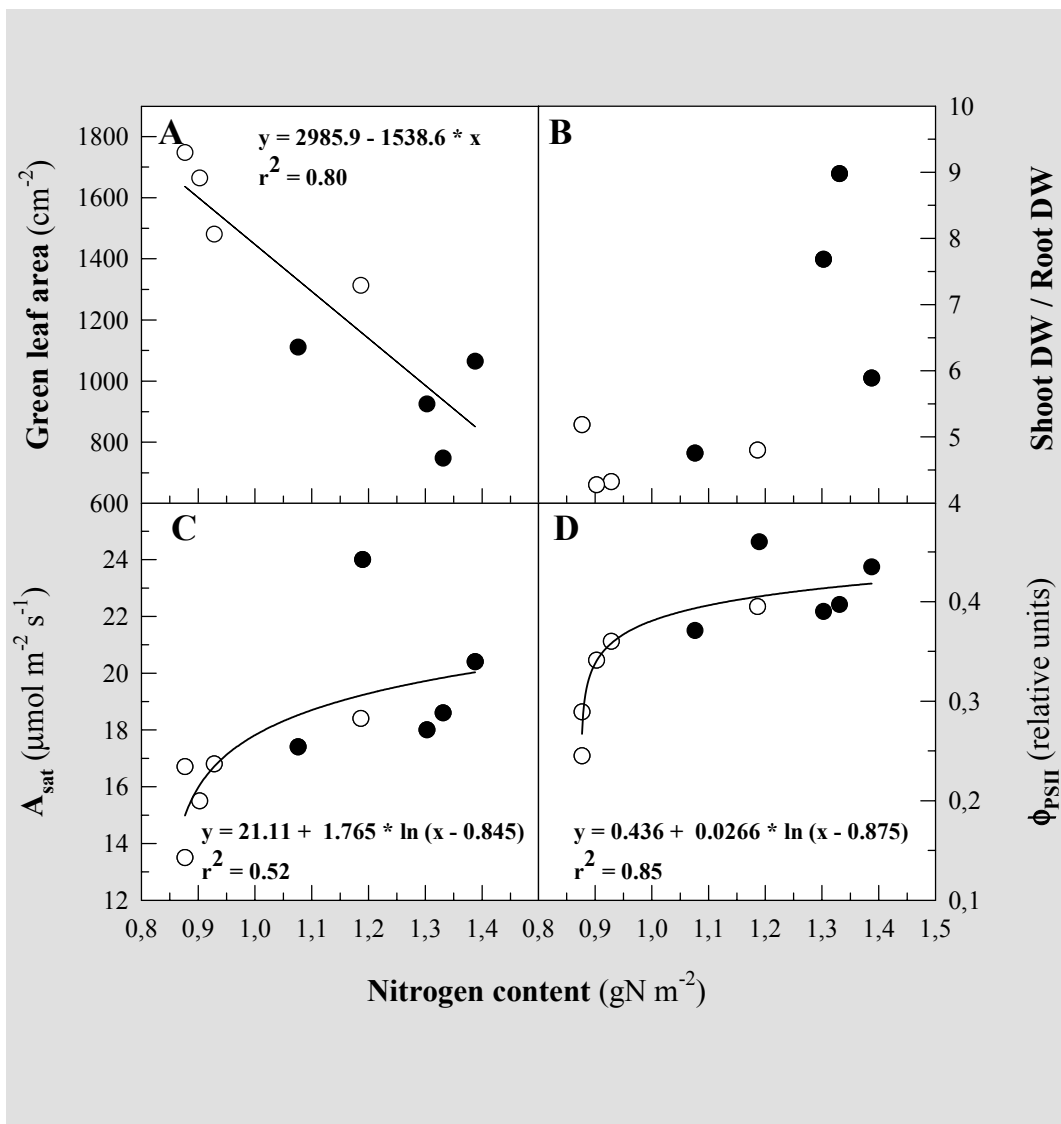


Figure 2. Relationship between leaf nitrogen content on area basis with (A) the total green area of the plant, (B) the shoot DW to root DW ratio, (C) the light-saturated net CO₂ assimilation rate (A_{sat}) on area basis and (D) the quantum yield of PSII electron transport (ϕ_{PSII}). Each point represents the value of an individual sample of the barley cultivars Graphic (○) and Kym (●) grown in a greenhouse for 8 weeks under optimal temperature and water conditions.

Quantum yield of photosystem II (ϕ_{PSII}) was also significantly higher in Kym than Graphic (Fig. 3B), this difference paralleling that of A_{sat} (Table 3). Moreover, this parameter also showed a positive relationship with nitrogen content, but for values over 1 gN m⁻² the

response was small (Fig. 2D). The lower ϕ_{PSII} in Graphic was associated with a lower photochemical quenching (qP) but not to a maximum quantum yield in dark-adapted leaves (F_v/F_m). The lower ϕ_{PSII} in Graphic (Table 3) indicates that the differences in A_{sat} between cultivars are also metabolic, rather than simply attributable to anatomical factors. The lower ϕ_{PSII} in this genotype was caused by a higher percentage of $PSII$ centres in a closed state (measured by qP) and not by intrinsic efficiency, because no difference was observed in the F_v/F_m' ratio (Table 3). Calvin cycle enzymes of the two cultivars may contribute to the differences in A_{sat} . Although Graphic showed a smaller mean $V_{c,max}$ than Kym, differences were not significant. By contrast, a lower and significant $J_{max,RuBP}$ was found in Graphic (Table 3), indicating that $RuBP$ regeneration could be involved in plant response (Nogués and Baker 2000).

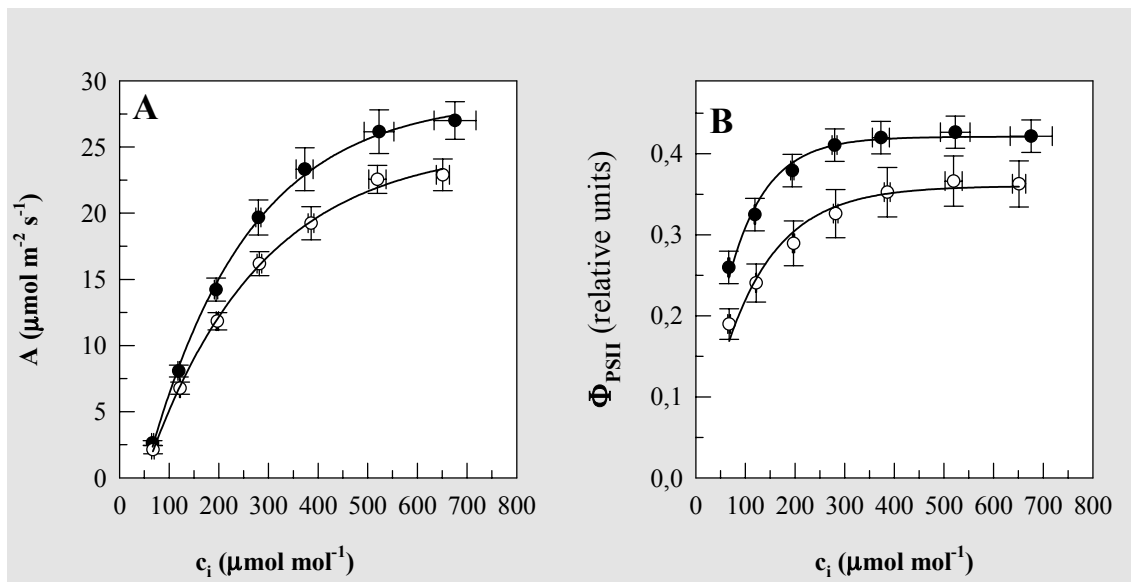


Figure 3. Effect of intercellular CO₂ concentration (c_i) on (A) the light-saturated net CO₂ assimilation rate (A_{sat}) and (B) the quantum yield of PSII electron transport (ϕ_{PSII}) of barley cultivars Graphic (○) and Kym (●). Plants were grown in a greenhouse for 8 weeks under optimal temperature and water conditions. A_{sat} and ϕ_{PSII} were measured simultaneously in the same recently fully-expanded leaves at 1200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD and at 25 °C. Each point represents the mean \pm SE of five leaves.

In spite of the lower photosynthetic capacity per unit leaf area of Graphic, stomatal limitation (l) was similar in the two genotypes (Table 3). Stomatal conductance tended to be lower (and transpiration, TR , was significantly lower) in Graphic. Consequently, the c_i and WUE (measured as the instantaneous A_{sat}/TR) was identical for the two genotypes (Table 3).

Similarly, there were no differences in $\Delta^{13}\text{C}$ of the leaf dry matter between cultivars, which indicates similar *WUE* over time (Hubick and Farquhar 1989).

7.4. Conclusions

Our case study support the view that selecting for improved yield potential improve yield in a wide range of Mediterranean environments. In other words, the crossover is not an universal phenomenon and higher yields in optimal and harsh conditions are not mutually exclusive. Graphic grew faster than Kym during tillering, even when its photosynthetic capacity per unit leaf area was lower, which is linked to lower nitrogen content and a concomitant decrease in Calvin cycle activities and electron transport rate. In spite of the differences in photosynthetic capacity, Graphic and Kym showed a similar leaf *WUE*.

Constitutive differences between genotypes in plant growth and leaf photosynthetic capacity during tillering are related to leaf nitrogen content per unit area, and indicated that a low content may be an appropriate selection trait for breeding strategies aimed at increasing yield performance in Mediterranean conditions.

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