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Photosynthetic limitations and yield potential in two barley genotypes (*Hordeum vulgare* L.) subjected to drought

Tambussi E A [1], Nogués S [1], Voltas J [2] and Araus J L [1]

[1] *Unitat de Fisiologia Vegetal, Facultat de Biologia, Universitat de Barcelona, Agda. Diagonal 645 (E-08028) Barcelona, Spain*

[2] *Departament Producció Vegetal i Ciencia Forestal, Universitat de Lleida, Rovira Roure 177 (25198) Lleida, Spain*

Introduction

Crop yield is a function of the interception of incident radiation, the efficiency of conversion in chemical energy (radiation-use efficiency or RUE) and the partitioning of the dry matter produced between the harvested parts and the rest of the plant (i.e. harvest index). Growth and yield of plants are severely reduced by water deficit, drought being the main abiotic stress limiting production of cereals and other major crops in Mediterranean conditions (Araus 2001).

Barley is one of the most widely cultivated cereals in the Mediterranean region where it occupies the harsher rainfed areas (Voltas 1998). In barley, when drought is imposed around anthesis, the primary cause of reduced biomass is a decrease in the leaf area duration (explained by leaf senescence). In contrast, when water deficit is imposed early, decrease in growth rate is caused primarily by reduction in RUE (Jamieson et al. 1995). In the other hand, although the strength of the sink is the main factor in grain filling in cereals, in poor rainfed environments the grain yield is largely limited by carbohydrate supply (i.e. source limited) (Voltas 1998). Clearly, the assimilation of CO_2 could be important in grain yield, particularly in dry areas, where it is one of the primary processes affected by water stress. One of the earliest responses to drought is the stomatal closure, which limits CO_2 diffusion to chloroplasts (Cornic 2000). However, when drought is prolonged or severe, non-stomatal limitation, such as Rubisco inactivation (see references in Flexas and Medrano 2001), decrease in photophosphorilation (Tezara et al. 1999) and damage to thylakoid protein (Tambussi et al. 2000) might occurred.

The aim of this study was to analyse the photosynthetic response of two cultivars of barley grown in pots and subjected to water stress. These two genotypes have shown contrasting agronomic performance under a wide range of Mediterranean conditions during the past years (Voltas 1998). The final objective was to study the relationship between the photosynthetic response to of these genotypes to water stress under controlled conditions and its agronomic performance in the field.

Material and methods

Seedlings of two barley (*Hordeum vulgare* L.) cultivars (Graphic and Kym), were grown in 1L pots filled with peat:perlite:vermiculite (2:1:1 v/v) and fertilized with Hoagland's solution. Seedlings were cultivated in a greenhouse at the University of Barcelona. Mean day/night

temperatures and maximum photosynthetic flux density (PPFD) were 25 ºC, 15ºC and 1000 μmol m^{-2} s^{-1}, respectively. Water stress treatment was imposed in seven-week-old plants by withholding water for 15 days. In order to avoid direct water evaporation, the top of the pots was sealed using cellulose film. Control (irrigated) plants were maintained at container capacity throughout the experiment.

The stomatal and non-stomatal limitation of photosynthesis was assessed by leaf gas exchange and chlorophyll fluorescence measurements (Nogués and Baker 2000). Measurements were performed in the youngest fully expanded leaves, between 09:00 AM and 05:00 PM. Leaf gas exchange was measured using an IRGA system LI-COR 6400 (LI-COR Inc., Lincoln, NB, USA). Net CO_2 assimilation rate (*A*) and intercellular CO_2 (c_i) concentration were calculated according von Caemmerer and Farquhar (1981). *A/c_i* curves were made at 1200 μmol m^{-2} s^{-1} PPFD. The temperature of leaf chamber was maintained at 25 ºC throughout the measurement. Stomatal limitation (*l*), which is the proportional decrease in light-saturated net CO_2 assimilation attributable to stomata, was calculated according to Farquhar and Sharkey (1982) as [(A_0 - A_{sat})/A_0] x 100, where A_0 is the *A* at c_i of 360 ppm and A_{sat} is A at c_a of 360 ppm. Estimation of $V_{c,max}$ and J_{max} (maximum carboxylation velocity of Rubisco and maximum potential rate of electron transport contributing to ribulose 1,5-biphosphate regeneration, respectively) were made by fitting a maximum likelihood regression below and above the inflexion of *A/ci* response as reported elsewhere (McMurtrie and Wang 1993).

Steady-state modulated chlorophyll fluorescence of leaves was simultaneously measured du*r*ing gas-exchange measurements with a fluorimeter MiniPAM (Walz, Effeltrich, Germany). The fluorescence signals were analysed as previously described (Nogués and Baker, 2000) to provide estimates of the relative quantum yield of PSII (ϕ_{PSII}, given by [F_m' - F_s]/F_m'), the intrinsic efficiency of PSII (F_v'/F_m', given by [(F_m'-Fo')/F_m']) and the photochemical quenching (q_P, given by [(F_m'-F_s)/(F_m-F_o')]). The potential quantum yield of PSII (F_v/F_m, given by [($F_m - F_0$) / F_m]) was determined in leaves after 15 minutes of dark adaptation. The parameter F_o' (minimum fluorescence yield in the light-adapted state) was estimated according to Oxborough and Baker (1997).

Leaf water potential (ψ_w) was determined using a pressure chamber (ARIMAD-2, ARI Far Charuv-Water Supply Accessories, Israel) with a damp paper at the bottom of the chamber to avoid excessive evaporation during the measurements. For relative water content (RWC) measurements, leaf segments were weighted (wi), floated on distilled water at 4°C overnight, weighted again (wf), dried at 80°C for 48 h, after which dry mass was determined (wd). Relative water content was calculated as: RWC = (wi - wd) (wf - wd)$^{-1}$ x 100. Water potential and RWC determination were made in similar leaves to those utilised for photosynthesis and fluorescence measurements. At the end of the drought treatment, plants were harvested and oven dried at 80 ºC and analyses of biomass of shoots and roots were carried out. Total leaf area was estimated prior to drying using a scanner and analysed with an image-processing program (Nogués and Baker 2000).

Field experiments were carried out in Lleida (Spain) during several years in three sites with contrasting water regime. Growing condition and experimental design are detailed elsewhere (Voltas 1998). Plants were harvested at maturity and yield was then calculated. Kernel were oven-dried and ground to a fine powder. The $^{13}C/ ^{12}C$ ratio was determined by mass spectrometry (Araus et al. 1997). Results were expressed as $\delta ^{13}C$ (‰) values where: $\delta ^{13}C$ (‰) = [($R_{sample}/R_{standard}$) - 1] x 1000 and R is the $^{13}C / ^{12}C$ ratio. The standard for comparison was a secondary standard calibrated against Pee Dee Belemnite (PBD) carbonate. Carbon isotope discrimination (Δ) was then calculated in kernels from δ_a and δ_p, where *a* and *p* refer to air and plant, respectively: $\Delta = (\delta_a - \delta_p) / (1 + \delta_p)$. The value of $\delta ^{13}C$ for the air was assumed to be -8.00 ‰ (Farquhar et al. 1989).

Results and discussion

The cultivar Kym showed a higher photosynthetic rate per unit leaf area than Graphic in non-stressed conditions. The Graphic cultivar had higher biomass and total leaf area. In cereals, a negative relationship between leaf area and photosynthetic rate has been reported by several authors (see references in Austin 1999) although in some case the correlation was very weak (Del Blanco et al. 2000). In wheat, this negative correlation has been adjudicated to the increase of the leaf width and/or a higher mesophyll cell volume in genotypes with small (and narrow) leaves (Austin 1999). In this sense, both genotypes did not shown differences in SLA, clearly showing that the difference in A_{sat} cannot be simply explained by leaf width. Consequently, other factors such as stomatal and/or non-stomatal limitations could be implicated. Analysis of the A/ci curves is a widely used method for discriminate stomatal and non-stomatal limitation in photosynthesis (Escalona et al. 1999; Nogués and Baker 2000). In well-watered plants, non-stomatal limitations were observed in cultivar Graphic, since $J_{max, RuBP}$ was lower in Graphic than in Kym. In addition, $V_{c, max}$ also showed differences, but there were not significant (no difference was observed between Graphic and Kym in l). This results suggests that some Calvin-cycle enzymes (different to Rubisco) could be implicated in the lower photosynthetic rate of Graphic. Accompanying the differences in A_{sat}, the actual quantum yield of the PSII (ϕ_{PSII}) was lower in Graphic, suggesting that the difference in A_{sat} between the cultivars could be "intrinsic", and not caused by anatomy factors. The lower ϕ_{PSII} in Graphic was caused mainly by a higher percentage of PSII centres in closed state (measured by q_P) rather by an intrinsic efficiency, because no difference was observed in F_v'/F_m' ratio.

The higher leaf area observed in Graphic was mainly associated to a higher leaves number, although difference in individual leaf size may not be discarded. Graphic also showed a higher total biomass and root dry weight, showing an enhanced growth capacity.

The decrease of ψ_w and RWC (between -1.4 and -1.65 MPa and 65-70 % respectively) of drougted-plants indicates that the water stress was from moderate to severe. The drop in water status parameters was stronger in Graphic, possibly explained by a higher transpiration rate (E) per plant. The transpiration rate per unit leaf area did not show significant differences, thus, the higher E per plant in Graphic cultivar was caused by a larger total leaf area.

Water stressed barley cultivars showed a decrease of light-saturated net CO_2 assimilation rate (A_{sat}). Analysis of A/ci curves showed that stomatal and non-stomatal limitations were found in water stressed-plants, indicated by the increase in l and the decrease of $V_{c, max}$ and $J_{max, RuBP}$. Non-stomatal limitation in water-stressed plants has been discussed for several years in the literature (Tezara et al. 1999; Cornic 2000; see references in Flexas and Medrano 2001). One important aspect in the interpretation of A/ci curves has been the possibility of heterogeneous stomatal closure, which could lead to overestimation of ci. Stomatal patchiness has been intensely analysed in ABA-treated and desiccated leaves (Terashima 1992). For instance, cereals as barley and wheat have not got heterovaric leaves (i.e. their leaves do not have bundle sheath extensions), therefore, the possibility of occurrence of patchiness is considered as limited (Terashima 1992). In fact, patchiness has not been observed in water-stressed leaves of wheat by at least two independent methods (Gunasekera and Berkowitz 1992; Martin and Ruiz-Torres 1992). Therefore, we can assume that heterogeneous photosynthesis did not occur in water-stressed barley plants

The decrease on $V_{c, max}$ and J_{max} in severe drougted plants has been observed for several studies (Martin and Ruiz Torres 1992; Nogués and Baker 2000). Although another causes may not be discarded (such us an increase in mesophyll resistance; Flexas and Medrano 2001), the drop in $V_{c,max}$ could be indicative of loss and/or inactivation of Rubisco. On other hand, the drop in $J_{max, RuBP}$ indicates that the decrease in photosynthetic capacity of water-

stressed plants was accompanied by a reduction in the ability to regenerate RuBP. RuBP regeneration could be limited either an inability to supply reductans and ATP from electrons transport or an inactivation or loss of Calvin cycle enzymes' (Nogués and Baker 2000). In this sense, the rate of PSII electron transport at saturating light and CO_2 ($J_{max,PSII}$) showed a similar decrease (ca. 60%) respect to $J_{max, RuBP}$.

The actual quantum yield of PSII showed a decrease (ca. 50%) in water-stressed plants. Both components of ϕ_{PSII} (i.e. q_P and F_v'/F_m') decreased in water-stressed plants, showing that there was both a reduction in percentage of open PSII centres and increase in thermal dissipation at antenna level. The drop in ϕ_{PSII} was lower than A_{sat}, therefore, ϕ_{PSII}/A ratio increased respect to controls plants. Several processes, such as photorespiration (Wingler et al. 1999) and/or Mehler's reaction (Foyer et al. 1994), could act as alternative sinks for electron transport. Maximum quantum yield of PSII (F_v/F_m ratio in dark-adapted leaves) did not show any changes between treatments, showing that the drop in ϕ_{PSII} could be due to down-regulation of electron transport rather than photodamage of photosynthetic apparatus. This observation is in agreement with several reports, which show that PSII is very resistant to water stress (Cornic 2000, Wingler et al. 1999).

Graphic and Kym showed similar responses to water treatments, although in the former the water deficit was higher. However, this observation can be explained by the higher transpiration rate per plant observed in Graphic. Interestingly, Graphic showed higher grain yield in field experiments and at least in two assays showed a superior carbon isotopic discrimination (Δ) in grains. This results shows that the better agronomic performance of Graphic could be related with a higher (integrated) stomatal conductance throughout the crop cycle (Araus 2001). This cultivar has also a higher biomass production despite the photosynthetic rate per unit leaf area. Several factors (such as a lower respiration rate) could be implicated. This hypothesis warrants further investigation.

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