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Grain number determination under contrasting radiation and nitrogen conditions in 2-row and 6-row barleys

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Abstract. Crop growth and developmental rate around the pre-heading phase are important for determining grain yield potential in barley (*Hordeum vulgare* L.) and other crop cereals. The photothermal quotient, Q (ratio between photosynthetically active radiation (PAR) and temperature) around the flowering period has been found to be a good predictor of grain number per unit area under potential growing conditions when both solar radiation and temperature vary, but not under suboptimal nitrogen (N) conditions. Under suboptimal conditions, Q might not account for differences in grain number due to modifications in radiation-use efficiency (RUE), biomass partitioning between vegetative and reproductive organs, fruiting efficiency, and/or a combination of these factors. This paper aims at providing insights into how grain yield is defined during the pre-heading phase in 2- and 6-row barleys under contrasting N and radiation environments, using a model proposed by RA Fischer for grain number determination.

Nitrogen and radiation treatments affected grain number, and consequently grain yield, through changes in spike biomass at heading, and not by a direct N effect. When low and high N conditions were included, Q poorly explained variations in grain number. Nitrogen increased RUE during the pre-heading phase. When accumulated PAR intercepted between the maximum number of spikelet primordia and heading stages (PAR_{ia}) was considered together with RUE, the accuracy of the model was increased. Nitrogen slightly increased biomass partitioning between reproductive and vegetative organs, but it was not strong enough to improve the model between PAR_{ia} and grain number. In the case of fruiting efficiency, genotype × N and shading × N interactions highlighted that this trait was maximised when 6-rowed barleys and shading were imposed under the high N treatment.

Additional keywords: biomass partitioning, malting barley, nitrogen, pre-flowering period, radiation.

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Introduction

In barley (*Hordeum vulgare* L.), as other cereals, variations in grain yield are mainly explained by changes in grain number per unit area rather than other yield components (Ramos *et al*. 1982; García del Moral *et al*. 2003; Prystupa *et al*. 2004; Arisnabarreta and Miralles 2006*a*). In wheat (Fischer 1985; Savín and Slafer 1991; Abbate *et al*. 1997), as well as in barley (Arisnabarreta and Miralles 2008*a*), grain number per unit area is established during the phase immediately pre-heading, when the spike and the stem are actively growing (Stockman *et al*. 1983; González *et al*. 2003*b*; Arisnabarreta and Miralles 2006*b*). Under potential growing conditions, greater amounts of accumulated assimilates allocated to the spike during the pre-heading phase have a strong impact on the establishment of the number of fertile florets and grains per unit area (Slafer *et al*. 1994; Miralles *et al*. 1998, 2000; González *et al*. 2003*a*; Prystupa *et al*. 2004; Arisnabarreta and Miralles 2008*b*, 2010). Therefore, any stress altering the crop physiological status (i.e. growing conditions below the potential) during that phase has an important negative impact on grain number per unit area, and thereby on yield, more so than during other phases during the crop cycle (Fischer 1985, 1993). This is why strong positive associations between grain number and photothermal quotient, Q (i.e. the ratio between photosynthetically active radiation (PAR) intercepted by the crop and temperature during the pre-flowering period, being the variables associated with crop growing conditions and rate of development, respectively; Fischer 1985) have been described in bread and durum wheat (Fischer 1985; Savín and Slafer 1991; Magrin *et al*. 1993; Abbate *et al*. 1995; Cossani *et al*. 2009), barley (Cossani *et al*. 2009) and triticale (Estrada-Campuzano *et al*. 2008).

The relationship between grain number and Q is not accurate under non-optimum crop-growing conditions (Abbate *et al*. 1995; Cossani *et al*. 2009). Some factors under contrasting nitrogen (N) conditions might explain the lack of fit between

Q and grain number. Under N limitation, the number of shoots per m^2 is reduced, with consequent negative impact on leaf area index, reducing the radiation intercepted by the crop (Fischer 1993; Abbate *et al*. 1995; Boonchoo *et al*. 1998; Caviglia and Sadras 2001). On the other hand, N limitations also diminish radiation-use efficiency (RUE) (Sinclair and Horie 1989; Abbate *et al*. 1995; Caviglia and Sadras 2001). Although Q accounts for changes in intercepted radiation, it cannot capture the variations in RUE due to changes in N availability. Under contrasting radiation and N supplies, Abbate *et al*. (1995) demonstrated that grain number per unit area was better predicted by spike N content than by spike biomass at anthesis, highlighting not only the importance of spike dry weight, but also the quality in terms of N. This statement was reinforced by Sinclair and Jamieson (2006) but lately rebutted by Fischer (2008), who demonstrated a lack of correlation between N concentration in spikes and the number of grains per unit of spike dry weight at anthesis. Differences in biomass partitioned to the growing spikes and the impact on grain number were also observed under contrasting N conditions in wheat (Demotes-Mainard *et al*. 1999). In addition, 2- and 6-row barleys show different behaviour under contrasting growing conditions around the pre-flowering phase, because radiation and N restrictions affect floret development, survival and main yield components (i.e. number of grains per spike and spikes per unit area) (Arisnabarreta and Miralles 2006*a*; Arisnabarreta and Miralles 2010) differently in those genotypes. Thus, variations in grain number under contrasting N conditions were associated with a higher plasticity to generate spikes per m^2 and grains per spike in 2-row and 6-row barleys, respectively (Arisnabarreta and Miralles 2006*a*).

The analyses of this paper used the following model of grain number (GN) determination in wheat proposed by Fischer (1984) as follows:

$$GN = SDW_{HD} \times FE_{HD} \quad (1)$$

$$SDW_{HD} = Ds \times CGR \times Ps \quad (2)$$

$$CGR = (PAR_{ia} \times RUE)/Ds \quad (3)$$

where SDW_{HD} is the spike dry weight at heading; FE_{HD} is the fruiting efficiency, i.e. number of grains per unit spike dry weight at heading; Ds is the duration of the spike growth period, which in barley is defined between maximum number of spikelet primordia (MNP) and heading (HD) stages (Arisnabarreta and Miralles 2006*b*); CGR is the crop growth rate around the spike growth period; Ps is the proportion of dry weight partitioned to the spike; and PAR_{ia} is the accumulated PAR intercepted between MNP and HD. In wheat and barley crops, Q might not be accounting for differences in RUE, Ps, FE_{HD}, and/or in a combination of these factors under contrasting N and radiation environments.

This paper aims at providing insights about how grain yield is defined around the pre-heading phase in 2- and 6-row barleys under contrasting N and radiation environments by using the model proposed by Fischer (1984) for grain number determination.

Materials and methods

General conditions and experimental design

The experiment was carried out under field conditions during the 2003 growing season in the experimental field of the Department of Plant Production, University of Buenos Aires (34°35′S, 58°29′W) and consisted of a factorial combination of two N fertiliser rates, two radiation levels and two near-isogenic barley lines (NILs). The NILs used, 106 2R (2-row, *Vrs1*) and 106 6R (6-row, *vrs1*), were chosen because they were expected to be identical at >99% of loci excluding the lateral spikelet fertility gene (*Vrs1*) and had shown contrasting behaviour under shading during the critical period for yield establishment (Arisnabarreta and Miralles 2008*a*). The pedigree was DAMPIER//(A14)PRIOR/YMER/3/KRISTINA (70S20-20)/4/ ARITMONT. Seeds were provided by the Australian Winter Cereal Collection.

The genotypes were hand-sown, with biodegradable paper adhesive tapes used to minimise variability in plant-to-plant distance and sampling errors. Sowing was at high density (880 seeds m^{-2}) to promote main-stem contributions in both barley types because they have different tillering capacities (Arisnabarreta and Miralles 2006*a*) and was done on 10 July 2003 in micro-plots in large cylindrical containers (200 L, 0.9 m height, 0.57 m diameter) in 7 rows 0.09 m apart oriented in a north–south direction. In order to ensure low N availability, the containers were filled with sand : soil mixture (3 : 1 by volume). The containers were perforated at the bottom and gravel was added in the lower 0.1 m for improving water drainage.

One week before sowing, soil samples were taken to determine N and phosphorous (P) concentration. Chemical soil analysis at that time showed 24 kg N ha^{-1} accumulated in the top 0.6 m soil layer and 9 kg P ha^{-1} (Bray and Kurtz 1945) in the top 0.2 m soil layer. Phosphorus was added to the soil before sowing at a rate of 10 kg P ha^{-1}as triple superphosphate. In addition, soil samples were extracted from the 0–0.3 and 0.3–0.6 m soil layers to determine N concentrations during the crop cycle. One week after seedling emergence, plots were thinned to a final density of 740 seedlings m^{-2}. Fungicides and insecticides were applied to prevent diseases and pests, and weeds were manually removed throughout the growing season.

Two radiation levels (unshaded control and shaded) were imposed during the active spike growth phase from MNP to HD (~20 days duration; Arisnabarreta and Miralles 2006*b*). The shaded treatment was achieved by installing black plastic nets 0.1 m above the crop canopy, reducing 68% ± 0.5% the incoming incident radiation measured at midday.

Nitrogen treatments were a control with the accumulated 24 kg N ha^{-1} available at sowing (N_{24}), and a treatment fertilised with ammonium nitrate with 210 kg N ha^{-1} added throughout the crop cycle with the irrigation in order to reach a total equivalent of 234 kg N ha^{-1} (N_{234}) (i.e. 24 + 210 kg N ha^{-1}). To impose the N treatments, two solutions containing macro- and micronutrients were made and mixed with the irrigated water. A first solution containing 6.4 mM K_2SO_4 as macronutrient, and 867 μM $MgCl_2$, 228 μM $ZnCl_2.4H_2O$, 664 μM $MnCl_2.4H_2O$, 86 μM $CuCl_2.4H_2O$, 118 μM H_3BO_3, 12 μM $CoCl_2.6H_2O$, 9 μM $MoO_4Na_2.2H_2O$ and 1547 μM $FeCl_3.6H_2O$ as micronutrients was used to impose the N_{24} treatment. A second solution was made with the same macro- and micronutrient

concentrations, plus 2.6 mM NH_4NO_3 as macronutrient, and was used to impose the N_{234} treatment (Arisnabarreta and Miralles 2010; Robson *et al*. 1995). Each container was irrigated every 3 days from sowing until beginning of stem elongation (BSE, Z31 Zadoks *et al*. 1974), and every 2 days from this stage until maturity with the corresponding solution.

Treatments were arranged in a completely randomised design with three replicates per treatment. Analyses of variance were performed by general linear model routines to determine the treatment effects on crop traits using the STATISTIX program. Significance among means were compared using the least significance differences test (l.s.d., $\alpha = 0.05$). Relationships between variables were made by linear or multiple regressions (Anon. 1991).

Measurements and analyses

Three plants per experimental unit were taken every 2 days from emergence to HD to establish the different ontogenic stages and to measure the dynamic of floral primordia initiated and mortality according to the scale proposed by Waddington *et al*. (1983). MNP was assumed when awn primordia started their elongation from the mid-third spikelet positions within the spike (García del Moral *et al*. 1991).

After determining the ontogenic stages, each sample was divided into different organs (i.e. stems plus sheaths, leaves blades and spikes) and oven-dried at 65°C until constant weight and then weighted. Nitrogen concentration was determined by the micro-Kjeldahl method (Bradstreet 1940). Organ N content was estimated multiplying the dry matter of each organ by its N concentration. Therefore, the dynamic of reproductive (i.e. spikes) and vegetative (i.e. stems, leaf blades and sheaths) dry matter and N accumulation was estimated throughout the crop cycle. Spike and stem dry matter accumulation and spike : stem ratio was estimated until HD and fitted with a bi-lineal model (González *et al*. 2003*a*):

$$Y = a + bx(x \leq c) + bc(x \geq c) + d(x - c)(x > c) \qquad (4)$$

The model considers two stages, an initial phase with a lower linear rate of spike or stem dry weight and a second phase with a higher linear rate of spike or stem dry weight, which are separated by an inflection point (IP). The parameters of biological interest were: *Y*, the spike and stem dry matter or spike : stem ratio; *b*, the minimum linear rate of spike or stem dry weight (and its ratio); *x*, days from heading; *c*, the timing of the IP, when the spike, stem or the spike : stem ratio began to grow at a maximum rate; and *d*, the maximum linear rate of spike and stem dry weight or its ratio. As parameter *b* was not different from zero in all treatments, it is not shown in the results. The parameters of the model were compared with confidence intervals (95%).

Crop and spike growth rates (g m^{-2} day^{-1}) during the active spike growth phase (~20 days before HD until HD) (Arisnabarreta and Miralles 2006*b*) were determined by linear regressions between the cumulative dry matter and days (Anon. 1991).

Heading time was assumed when 50% of the plants within each plot exposed the total spike above the flag leaf. Plant samples were taken at HD to measure aerial biomass from two 0.3-m linear central rows (maintaining the adjacent rows as a border), dried until constant weight, and weighted. Three main shoot spikes per replicate were taken at HD to measure the number of fertile florets per spike. A floret was considered alive if it had reached a development stage of $W_{9.5}$ (i.e. green anthers visible, pollen grains on stigmas and stigmatic branches well developed; Waddington *et al*. 1983).

Air temperature was measured (*i*) hourly in the field at 1.5 m using a meteorological station (Weather Monitor II, Davis Instruments, Hayward, CA USA), and (*ii*) at the level of shoot apex from MNP to HD (i.e. during the shading treatment period) within each shading treatment. The difference in the mean air temperature during the shading period, between shaded and control treatments, was <1°C.

From MNP to HD, incident and transmitted radiation were measured in clear days at noon ± 1 h with a linear radiometer (LI-191 S; LI-COR Inc., Lincoln, NE, USA). The percentage of intercepted radiation (IR %) was estimated as:

$$IR\% = 100 \times (1 - (I/I_0)) \qquad (5)$$

where, *I* is the incident and transmitted radiation measured immediately above the senesced leaf layer, and I_0 is the incident radiation measured above the crop canopy. The changes with time of intercepted radiation for each treatment were fitted with a sigmoid function (Anon. 1991). The PAR intercepted by the crop (PAR_i) (MJ m^{-2} day^{-1}) was calculated each day as the product of IR%, incident radiation (MJ m^{-2}) and 0.48 (i.e. ratio of PAR to total radiation; Szeicz 1974). Incident radiation was measured each hour with a standard meteorological station 200 m from the experimental field. The accumulated PAR intercepted between MNP and HD (i.e. PAR_{ia}) (MJ m^{-2}) was calculated as the daily sum of PAR_i between MNP and HD. RUE (g MJ^{-1}) was estimated as the ratio between the accumulated biomass (g m^{-2}) and PAR_{ia} between MNP and HD. The mean Q during MNP and HD was calculated as the ratio between PAR_{ia} and the daily sum of the difference between mean air (T_{mean}) and base (T_{base}) temperature estimated at 4.5°C (Fischer 1985; Savín and Slafer 1991; Cossani *et al*. 2009).

At harvest (HV), plant samples from two 0.3-m linear central rows were taken and dried until constant weight. Grain yield and its numeric (i.e. grain number per m^2 and mean grain weight) and physiological (i.e. aboveground biomass and harvest index (HI)) components determined. In order to avoid edge effects from samplings at HD to HV, at least one row without altered crop structure was left between sample times. Grain setting was estimated as the ratio of number of grains per spike to number of fertile florets per spike. FE_{HD} was calculated as the ratio of grain number per spike obtained at harvest and spike dry weight at heading.

Results

Weather conditions

Temperatures during the 2003 growing season were higher than registered in the long-term records (1970–2003) (Fig. 1). On the other hand, monthly mean solar radiation for the 2003 growing season was slightly lower than recorded for the long-term from emergence to MNP, but similar for the following crop phases (Fig. 1).

Above ground crop nitrogen dynamics

Soil N content throughout the crop cycle was different in the N_{24} and N_{234} treatments (Fig. 2 Inset). Nitrogen availability in the soil

was lower in N_{24} than the N_{234} treatment at 41 days after sowing (around double-ridge stage). However, from about MNP onwards, soil N was similar in both N treatments.

Aboveground crop N dynamics differed depending on N and shading treatments. From BSE onwards, crop N increased sharply, and differences in aboveground crop N content between treatments were magnified. These differences were even greater when N_{24} and N_{234} were compared in the non-shaded treatment (Fig. 2). Aboveground crop N content was lower in the shaded than the control treatment under higher N supply; however, these differences were not observed under N_{24}. At HD, aboveground crop N concentration values were similar ($P > 0.05$) when radiation ($1.88\% \pm 0.18\%$ and $1.97\% \pm 0.15\%$ in the control and shaded treatment, respectively) and N ($1.90\% \pm 0.19\%$ and $1.95\% \pm 0.14\%$ in N_{24} and N_{234}, respectively) treatments were compared.

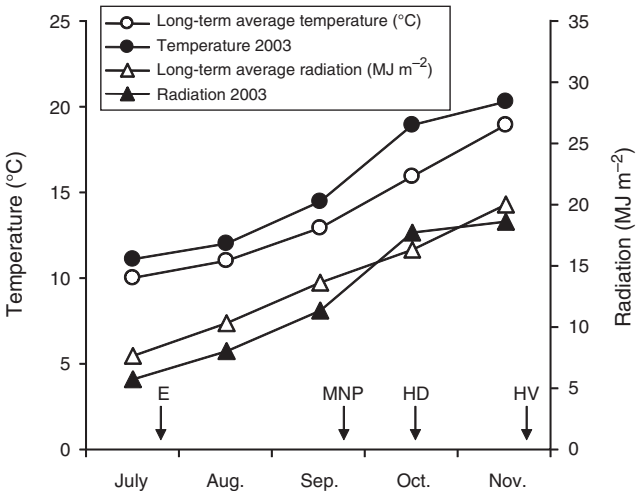

Fig. 1. Long-term (1970–2003) average temperature and radiation, and mean temperature and radiation for the 2003 growing season. Arrows indicate the time to emergence (E), maximum number of spikelet primordia (MNP), heading (HD) and harvest (HV). Values are the mean for the different treatments.

Grain yield and its components

Grain yield was more affected by N shortage (60% reduction in N_{24} compared with N_{234}, $P < 0.001$) than by shading treatments (23% reduction in shaded compared with unshaded treatment; $P < 0.01$) (Table 1). The mean square errors for N were 10 times higher than for radiation treatments (data not shown). Considering the association between grain yield and the numerical yield components, a stepwise regression revealed that grain number per unit area had a greater contribution ($R^2 = 0.86$, $P < 0.001$) to variations in grain yield than mean grain weight ($R^2 = 0.28$, $P > 0.05$). When both numerical components were included in the analyses, the model improved significantly over that with grain number per unit area ($R^2 = 0.99$, $P < 0.001$). Independent of shading and/or N treatments, the 6-row barley had a higher grain number per m^2 than the 2-row barley. These differences between genotypes were offset by a lower mean grain weight in the former than in the latter,

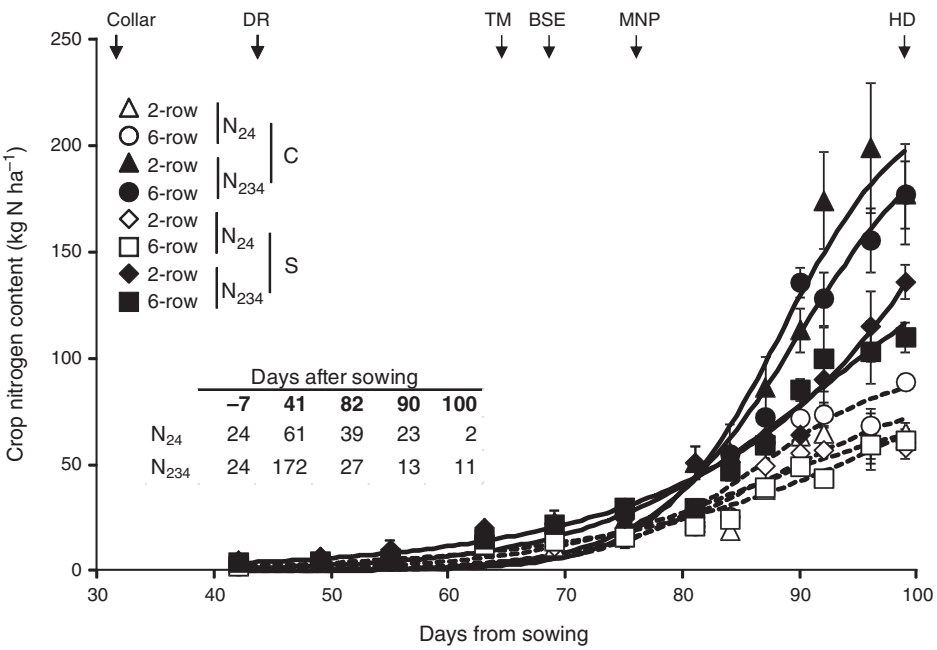

Fig. 2. Above-ground crop nitrogen content dynamics (kg N ha^{-1}) for the 2-row and 6-row barleys, in the control (C) and shaded (S) treatments and the low (N_{24}) (- - -, open symbols) and high (N_{234}) (—, closed symbols) N supply treatments. Arrows indicate the stages of collar, double-ridge (DR), triple-mound (TM), beginning of stem elongation (BSE), maximum number of spikelet primordia (MNP) and heading (HD). Values are the mean for the different treatments. Inset: soil mineral N content (kg N ha^{-1}) for different sample times throughout the crop cycle.

Table 1. Grain yield (GY, kg ha^{-1}), grain number per m^2 (GN), mean grain weight (GW, mg), grain setting (GS, ratio of number of grains per spike to number of fertile florets per spike), aboveground biomass at maturity (AGB, kg ha^{-1}), harvest index (HI, ratio of grain yield to AGB) and fruiting efficiency (FE_{HD}, number of grains per unit spike dry weight at heading) in the 2-row and 6-row near-isogenic lines under the nitrogen (N_{24} and N_{234}) and shaded (C, control; S, shaded) treatments

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; n.s., not significant. Values in parentheses are l.s.d.

Genotype	Radiation	Nitrogen	GY	GN	GW	GS	AGB	HI	FE_{HD}
2-row	C	N_{24}	3351	8615	38.8	0.97	7156	0.47	132.9
		N_{234}	7560	16 933	44.5	0.80	15 731	0.48	80.0
	S	N_{24}	2155	6162	33.6	0.83	5042	0.42	138.6
		N_{234}	5897	14 347	41.1	1.02	12 727	0.46	127.0
6-row	C	N_{24}	2691	8572	31.5	0.60	6020	0.45	111.0
		N_{234}	6869	21 738	31.7	0.63	13 398	0.51	120.1
	S	N_{24}	2195	7592	29.6	0.58	5648	0.38	140.9
		N_{234}	5439	17 283	31.3	0.85	11 806	0.46	192.1
				Main effects					
Genotype (G)									
2-rowed			4741	11 515	39.5	0.91	10 164	0.46	119.6
6-rowed			4299	13 796	31.0	0.67	9218	0.45	141.0
Radiation (R)									
Control			5118	13 965	36.6	0.75	10 576	0.48	111.0
Shaded			3921	11 346	33.9	0.82	8806	0.43	149.6
Nitrogen (N)									
N_{24}			2598	7735	33.4	0.74	5966	0.43	130.8
N_{234}			6441	17 575	37.1	0.83	13 416	0.48	129.8
				Significant effects					
G			n.s.	* (2040)	***	*** (0.08)	n.s.	n.s.	*** (15)
R			** (764)	* (2040)	** (1.9)	n.s.	* (1304)	*** (0.02)	*** (15)
N			*** (764)	*** (2040)	***	*	*** (1304)	*** (0.02)	n.s.
G × R			n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
G × N			n.s.	n.s.	** (2.7)	n.s.	n.s.	n.s.	*** (22)
R × N			n.s.	n.s.	n.s.	** (0.11)	n.s.	n.s.	* (22)
G × R × N			n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

resulting in similar yields in both genotypes (Table 1). Lower N and radiation levels significantly reduced grain number per m^2 in both genotypes; however, there were no interactions between genotype, N treatment and shading treatment. Interestingly, shading during the MNP–HD phase also reduced grain weight; the reductions were substantially less than those recorded on grain number per unit area. Averaging both N treatments, mean grain weight was 41.7 and 37.3 mg in 2-row barley, and 31.6 and 30.5 mg in 6-row barley in the control and shaded treatments, respectively. There was a strong N × genotype interaction ($P < 0.01$) when grain weight was analysed, whereby 2-row barley showed greater response to increases in N availability than 6-row barley (i.e. mean grain weight was 36.2 and 42.8 in 2-row barley, and 30.5 and 31.5 mg in 6-row barleys in the N_{24} and N_{234} treatments, respectively) (Table 1).

Grain setting was lower ($P < 0.001$) in the 6-row (0.67 ± 0.14) than the 2-row (0.91 ± 0.12) barley (Table 1). There was a significant N × shading treatment interaction ($P < 0.01$), in which the relative impact of shading on grain setting was greater under the high than the low N treatment (Table 1).

Grain yield showed a significant and positive correlation with aboveground biomass at harvest ($P < 0.001$), and with HI ($P < 0.05$). However, the variation produced by shading and N treatments was 68% for aboveground biomass and 25% for HI (Table 1). Aboveground biomass and HI did not differ between genotypes; however, higher N or radiation levels significantly increased both physiological yield components.

Factors affecting grain number determination

The Q appropriate to each crop during the active spike growth phase (i.e. MNP–HD) weakly explained the changes in grain number per unit area when the whole dataset was fitted with a common linear regression ($R^2 = 0.5$, $P > 0.05$). When grain number per unit area was plotted against PAR_{ia} (Fig. 3; $R^2 = 0.4$, $P > 0.05$), the same trend was observed as when grain number per unit area and Q were plotted, highlighting that any difference in Q among treatments was mainly driven by changes in PAR_{ia}, rather than by any change in thermal time for the MNP–HD phase. When the relationship between grain number and PAR_{ia} was fitted for each N level, the correlation coefficient was improved ($R^2 = 0.7$ and $R^2 = 0.5$ for N_{24} and N_{234}, respectively), showing that grain number was always higher in N_{234} than in N_{24}, independent of the level of PAR_{ia} of the crop.

The CGR was significantly correlated ($R^2 = 0.81$, $P < 0.01$) with PAR_{ia}, when the whole dataset was considered (Fig. 4*a*). However, different trends were also observed within N treatments. Thus, and similar the relationship between grain number per unit area and PAR_{ia}, for the same value of PAR_{ia}, CGR was higher in N_{234} than in N_{24}, highlighting differences in RUE between N treatments (Fig. 4*a*). Genotype and N treatments

altered RUE ($P < 0.01$), whereas shading treatments did not ($P > 0.05$). Averaging among genotypes and shading treatments, RUE was 1.8 ± 0.4 and 3.1 ± 0.6 g biomass MJ^{-1} for N_{24} and N_{234}, respectively. Averaging among N and shading treatments, RUE values were 1.8 ± 0.5 and 3.0 ± 0.6 g biomass MJ^{-1} in the 2-row and 6-rowed barley, respectively (see table 1 in Arisnabarreta and Miralles 2010). Genotypes did not differ in PAR_{ia} during the MNP–HD phase; however, a significant shading × N treatment interaction ($P < 0.05$) was observed in PAR_{ia}, increasing 40% and 33% when the crop was fertilised in the control and shaded treatments, respectively (see table 1 in Arisnabarreta and Miralles 2010).

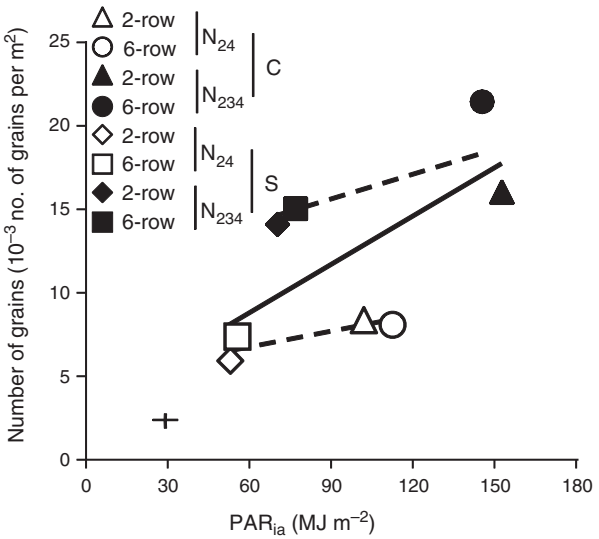

Fig. 3. Relationship between grain number per m^2 and photosynthetically active radiation intercepted during the active spike growth phase between the maximum number of spikelet primordia and heading stages (PAR_{ia}) for the 2-row and 6-row barleys, in the control (C) and shaded (S) treatments and the low (N_{24}, opened symbols) and the high (N_{234}, closed symbols) nitrogen supply treatments. Crossed vertical and horizontal lines are the standard error of the means. Dashed lines are the regressions within each N treatment. Solid line is the regression considering all treatments.

Independent of treatments, changes in spike growth rate (SGR) were closely associated with changes in CGR during the MNP–HD phase ($P < 0.001$) (Fig. 4*b*). Apparently, the stronger the stress, by reduced N or radiation levels, the higher the relative biomass partitioned towards reproductive organs (note that points with reduced CGR approached the 1 : 1 relationship and the positive intercept in Fig. 4*b*). In relative terms, SGR ($SGR_{shaded}/SGR_{control}$) was less affected than crop growth rate ($CGR_{shaded}/CGR_{control}$) when crop was shaded under N_{24} (see inset Fig. 4*b*).

Because parameter *b* (i.e. the initial slope preceding the inflection point in the dynamic of the spike : stem ratio) was not different from zero (see Fig. 5) and time of HD did not differ across treatments ($P > 0.05$), the spike : stem ratio at heading could be explained by the maximum linear rate of change of spike : stem dry weight ($S : S_{max}$) and its duration (i.e. IP–HD). Spike : stem ratio dynamics slightly changed because of N or radiation treatments (Fig. 5, Table 2). On one hand, in 2-row barleys the IP tended to be earlier in N_{234} than in N_{24}, although that behaviour was not evident in the 6-row barley. On the other hand, $S : S_{max}$ was higher in N_{234} than N_{24} only when radiation was not limiting in both genotypes (Fig. 5, Table 2).

The spike : stem ratio at heading increased as spike dry weight at the onset of the maximum stem growth rate increased (Fig. 6). However, when a higher dose of N was applied, it increased the value of the intercept without changes to the slope of the relationship between spike : stem ratio and the relative spike dry weight at the onset of the maximum stem growth rate,

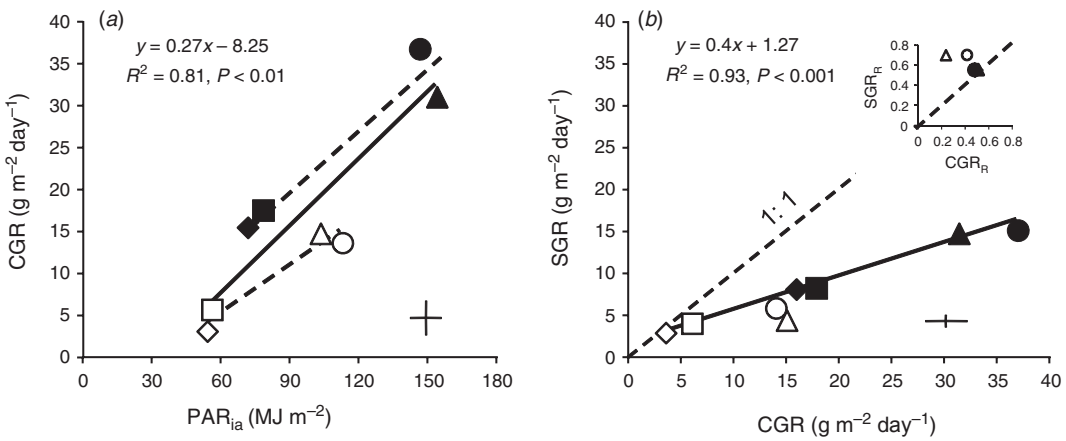

Fig. 4. Relationship between (*a*) crop growth rate and accumulated photosynthetic active radiation intercepted (PAR_{ia}) and (*b*) spike growth rate (SGR) and crop growth rate (CGR) during the active spike growth phase between the maximum number of spikelet primordia and heading stages. Symbols are: control, triangles and circles; shaded, diamonds and squares; N_{24}, open symbols; N_{234}, closed symbols; 2-row barley, triangles and diamonds; 6-row barley, circles and squares. Inset: relationship between relative SGR ($SGR_{shaded}/SGR_{control}$) and relative CGR ($CGR_{shaded}/CGR_{control}$) in the N_{24} (open symbols) and N_{234} (closed symbols) treatments in 2-row (triangles) and 6-row (circles) barleys. Crossed vertical and horizontal lines are the standard error of the means. The dashed lines in (*a*) are the regressions within each N treatment, and in (*b*) are the 1 : 1 relationships. Solid lines and equations are the regressions considering all treatments.

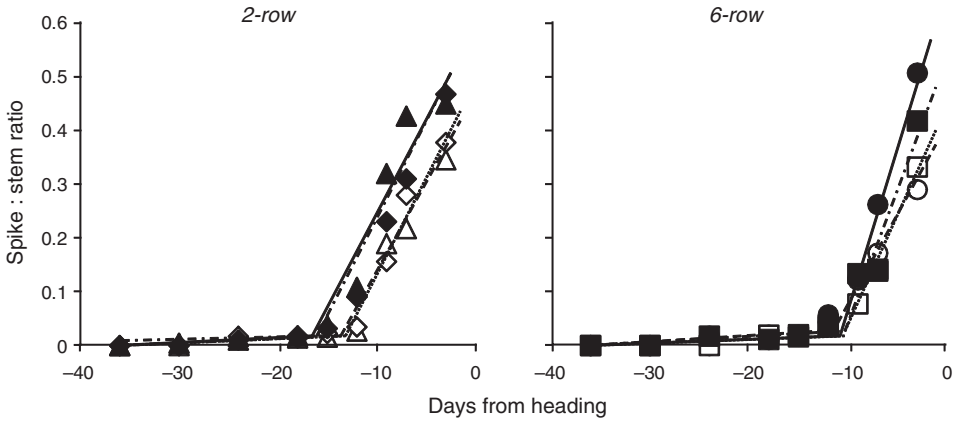

Fig. 5. Spike : stem biomass ratio dynamics for the 2-row and 6-row barleys, in the control (triangles or circles) and shaded (diamonds or squares) treatments, and the low (N_{24}) (open symbols) and high (N_{234}) (closed symbols) nitrogen supply treatments. The bi-linear regressions correspond to the high N control (—), high N shaded (– – –), low N control (- - -) and low N shaded (. . ..) treatments.

Table 2. Parameters derived from the bi-linear model for the relationship between spike : stem ratio and days from heading (see Fig. 6)
The parameters (± s.e.) are the inflection point (IP, days) and the maximum linear rate of change of spike to stem dry weight ($S : S_{max}$, $days^{-1}$) in the 2-row and 6-row near-isogenic lines under the nitrogen (N_{24}, N_{234}) and radiation (control, shaded) treatments. Between treatments within a barley type, values followed by the same letter are not significantly different ($P > 0.05$)

Radiation	Nitrogen	IP	$S : S_{max}$
		2-rowed	
Control	N_{24}	–13.1 ± 0.5ab	0.034 ± 0.002b
	N_{234}	–15.9 ± 1.0b	0.039 ± 0.004a
Shading	N_{24}	–12.8 ± 0.4a	0.039 ± 0.002a
	N_{234}	–14.0 ± 0.2ab	0.042 ± 0.001a
		6-rowed	
Control	N_{24}	–13.1 ± 0.2b	0.027 ± 0.001c
	N_{234}	–10.7 ± 0.3a	0.063 ± 0.003a
Shading	N_{24}	–10.2 ± 0.3a	0.043 ± 0.002b
	N_{234}	–10.5 ± 0.6a	0.051 ± 0.005ab

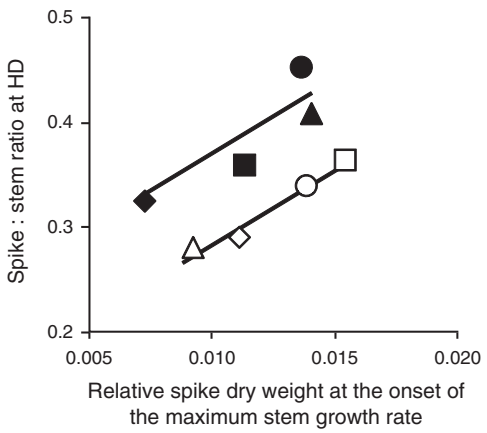

Fig. 6. Relationship between spike : stem biomass ratio at heading (HD) and relative spike biomass at the onset of the maximum stem growth rate (ratio of spike dry weight at the stem inflexion point to spike dry weight at heading) for the 2-row barley (triangles and diamonds) and 6-row barley (circles and squares), in the control (triangles and circles) and shaded (diamonds and squares) treatments and the low (N_{24}) (open symbols) and the high (N_{234}) (closed symbols) nitrogen supply treatments.

suggesting that N increased the efficiency of dry matter partitioning from the stem to the spike (at least in relative terms) (Fig. 6).

Changes in grain number per unit area were significantly correlated with spike dry weight at heading, independent of genotype, shading or N treatment (Fig. 7). In order to analyse a direct effect of N on this yield component, grain number per unit area was plotted against spike N content at heading (data not shown). The correlation coefficient, as well as the residuals distribution from the fitted relationship, did not improve compared with the relationship between grain number per unit area and spike dry weight at heading (see Fig. 7). As previously stated, treatments did not affect ($P > 0.05$) spike N concentration at heading, the range of variation being 1.8–2.2%. There was a significant genotype × N ($P < 0.001$) and shading × N ($P < 0.05$) interaction on FE_{HD}, showing that 2- and 6-row barleys did not differed in FE_{HD} under N_{24} but 6-row barley had a higher FE_{HD} than the 2-row barley under N_{234} (Table 1). In addition, radiation treatments did not differ under N_{24}, but shading significantly increased FE_{HD} under N_{234} (Table 1). Analysing the whole dataset, there was no relationship between FE_{HD} and spike N concentration at heading ($R^2 = 0.038$).

Discussion

Contrasting N and radiation conditions around the pre-heading phase altered numeric yield components (i.e. grain number per unit area and grain weight) in 2- and 6-row barleys. Similar to previous studies in barley (Ramos *et al*. 1982; García del Moral *et al*. 2003; Prystupa *et al*. 2004; Arisnabarreta and Miralles 2006*a*), changes in grain yield were more associated with changes in grain number than grain weight. Following the conceptual model of grain number determination previously described by

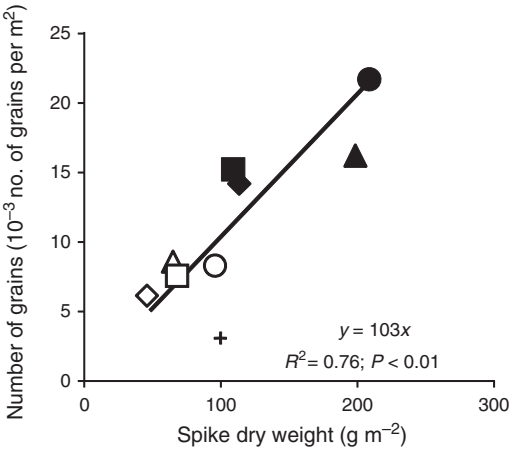

Fig. 7. Relationship between grain number per m^2 and spike dry weight at heading. Symbols are: control, triangles and circles; shaded, diamonds and squares; N_{24}, open symbols; N_{234}, closed symbols; 2-row barley, triangles and diamonds; 6-row barley, circles and squares. Vertical and horizontal lines indicate the standard error of the means.

Fischer (1984, 2008) (Eqns 1–3), it was possible to dissect how contrasting N and radiation levels affected this yield component. Thus, in the present paper, as well as in numerous previous studies (Stockman *et al*. 1983; Fischer 1993; Slafer *et al*. 1994; Miralles *et al*. 1998; González *et al*. 2003*b*; Prystupa *et al*. 2004; Arisnabarreta and Miralles 2008*b*), changes in grain number were mainly driven by changes in spike dry weight at heading or anthesis. Abbate *et al*. (1995) observed that grain number per unit area was better correlated with spike N content than spike biomass at anthesis. Nevertheless, Demotes-Mainard *et al*. (1999) and Fischer (1993), imposing different timings and amounts of N in wheat, observed no direct effect of N on grain number apart from the effect on spike dry weight. The results of the present paper suggest an indirect effect on grain number via a direct effect on spike weight, since low and high N treatments produced lighter or heavier spikes, respectively, but showed a common linear relationship with grain number per unit area. In addition, differences in the relationship between grain number and spike dry weight or spike N content at heading were negligible.

As described in Eqn 2, spike dry weight at heading can be explained as the product of the duration of the spike growth period, crop growth rate and partition to spikes (Fischer 1984). As reported by Arisnabarreta and Miralles (2010), the active spike growth period was not altered by N or radiation treatments. However, CGR during the spike growth period was enhanced by higher N and radiation levels in agreement with the reports by Abbate *et al*. (1995), Fischer (1993) and Lázaro *et al*. (2010). In turn, PAR_{ia}, and to a lesser extent RUE, were responsible for those changes in CGR (Fig. 4). For a given PAR_{ia}, the CGR was higher when crops were grown under high than low N treatments. In the present work, as in many other published works (e.g. Sinclair and Horie 1989; Hammer and Wright 1994; Abbate *et al*. 1995; Caviglia and Sadras 2001; Muurinen and Peltonen-Sainio 2006), the RUE during the spike growth period was affected by N supply, whereas shading did not affect this crop attribute.

In the present study, partitioning to spikes at heading was analysed through the spike : stem ratio and its components (i.e. the IP–HD time and the $S : S_{max}$). The results showed that biomass partitioned to the growing spike tended to increase from the low to the high N treatment. Similarly, Demotes-Mainard *et al*. (1999) observed a higher biomass partitioned to the spikes at anthesis in wheat under high N treatments. This work confirms that suboptimal growing conditions during the immediate pre-heading phase affected CGR, and consequently SGR. However, the latter was less affected than the former, reinforcing previous results in wheat (Fischer 1985; Abbate *et al*. 1995, 1997) where biomass partitioned to reproductive organs was increased, in relative terms, when crops were stressed during their active spike growth phases.

The results of the present study demonstrate that in barley the proportion of biomass partitioned to the spike was positively correlated with the size of the spike at the beginning of stem elongation, and that increases in N promoted a higher spike : stem ratio for the same spike dry weight at the beginning of stem elongation. González *et al*. (2011), analysing a wide dataset of wheat genotypes grown under different environmental conditions, demonstrated that the onset of floret death was associated with the beginning of the maximum spike growth rate; and the rate of floret death, which is the main determinant of floret survival, was negatively associated with spike weight at anthesis. Our results suggest that in barley the role of biomass partitioned to the spike at the beginning of stem elongation in the determination of grain number is similar to that observed in wheat.

Fruiting efficiency showed genotype × N and radiation × N interactions whereby, under high N availability, the differences among genotypes and radiation treatments were maximised. Additionally, FE_{HD} was significantly different between genotypes, with 6-row barley more efficient at setting grains per unit of spike dry weight at heading than 2-row barley. Our results also showed that shading increased FE_{HD} under the N_{234} treatment, in agreement with Abbate *et al*. (1995) and Lázaro *et al*. (2010), where shading during the spike growth phase in wheat significantly increased FE_{HD}. However, Fischer and Stockman (1980) observed that shading during the active spike growth phase in wheat reduced the ratio of grain number to chaff dry weight, the reduction being higher when shading intensity increased. In the present study, and in line with observations in wheat with contrasting N (Abbate *et al*. 1995; Fischer 1993) and P levels (Lázaro *et al*. 2010), N did not alter FE_{HD}. When FE_{HD} was plotted against spike N concentration, a non-significant correlation was found ($P > 0.1$). Similar results were obtained by Fischer (2008), who determined an R^2 of 0.077 between FE_{HD} and spike %N in wheat, suggesting no direct effect of N on FE_{HD}.

The results of the present work demonstrated that N increased (*i*) the PAR intercepted by the crop, (*ii*) the RUE, and (*iii*) the biomass partitioned to the spike during the critical period for yield determination (Arisnabarreta and Miralles 2008*a*), regulating in this way the grain number per unit area through spike biomass at anthesis. To determine whether the introduction of variables such as RUE and spike : stem ratio improves the fit of PAR_{ia} and grain number per unit area, stepwise regressions were done considering the previous sources of variation (Fig. 8). The highest R^2 values, between the actual grain number and the grain number predicted by the model were observed when PAR_{ia} and RUE, and PAR_{ia}, RUE and spike : stem ratio at heading were included in the model.

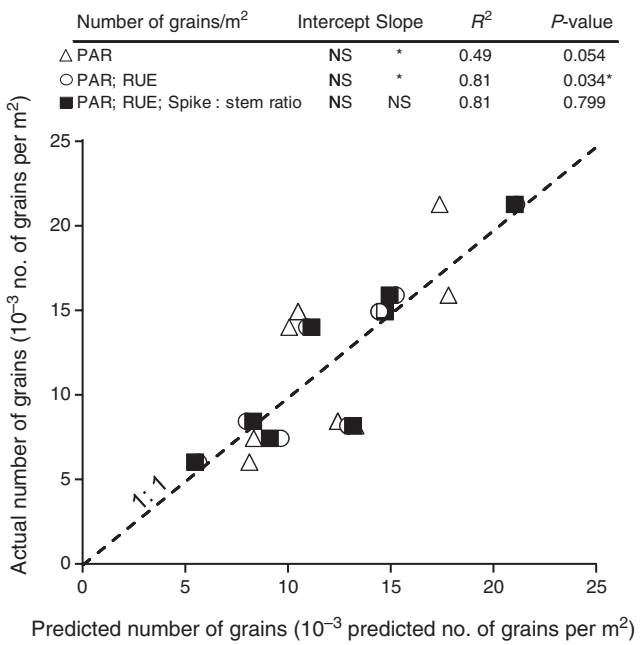

Fig. 8. Relationship between the actual and the predicted grain number per m^2 modelled using the photosynthetically active radiation intercepted during the active spike growth phase between the maximum number of spikelet primordia and heading stages, i.e. PAR_{ia} (PAR, △), PAR_{ia} and radiation-use efficiency (PAR; RUE, ○), and PAR_{ia}, RUE and spike : stem biomass ratio at heading (PAR; RUE; Spike : stem ratio, ■). The table indicates the R^2 and the *P*-value for the linear and multiple regressions. NS, $P > 0.05$; $*P < 0.05$, for the intercepts and the slopes for the linear regression between actual and predicted grain number per m^2.

However, spike : stem ratio did not seem to improve the value of the fitted regression. In fact, the lowest *P*-value in the stepwise regression analyses was found when only PAR_{ia} and RUE were considered, rather than any other combination of variables (Fig. 8). These findings are consistent with the effect of N observed here, because N impact on RUE was more than on spike : stem ratio.

Shading before the grain-filling period also affected grain weight, reinforcing the statement that grain number and grain weight determination are not completely separated in time. Floret primordia, fertile florets or even small grains in distal spikelets positions may abort with poor radiation conditions immediately before heading (Arisnabarreta and Miralles 2008*a*, 2010), reducing the contribution of grains with lower potential grain weight. In addition, potential grain weight at different spikelet and floret positions may be reduced by shading events at 1 week before anthesis (Calderini and Reynolds 2000).

In the present study, a significant genotype × N interaction was found on grain weight, highlighting that 2-row barley responded more to N fertilisation than 6-row barley. Following the approach of Fischer (1993), the assimilate supply to grain filling was calculated as the quotient between grain yield and the difference between total dry weight from 50% anthesis to maturity. In our study, this ratio was 115% and 112% in 2-row barley and 196% and 182% in 6-row barley under N_{24} and N_{234}, respectively, suggesting that post-anthesis assimilate supply seems lacking in 6-row genotypes and tends to be higher under high than low N supply. On one hand, these results contrast with those of Fischer (1993), who found that surplus assimilate supply to grain filling (i.e. <100%) tended to increase with N fertilisation, and on the other hand, they are in agreement with Borrás *et al*. (2004), who stated that wheat yield is mainly limited by sink size, although our results showed that 2-row barley was more limited by sink size than 6-row barleys.

Conclusions

Nitrogen affected grain number, and consequently grain yield, through effects on spike biomass at heading. This is in contrast to Sinclair and Jamieson (2006) and cites therein, which suggested that the relationship between the grain number per unit area and grain yield was merely casual, and the amount of N absorbed by the crop was the real driver altering grain number. Results from our study confirmed that the amount of carbon produced during the active spike growth phase and partitioned to the growing spike was the main driver explaining variations in grain number per unit area in barley.

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