CSIRO PUBLISHING
www.publish.csiro.au/journals/cp *Crop & Pasture Science*, 2010, **61**, 578–587

Nitrogen and radiation effects during the active spike-growth phase on floret development and biomass partitioning in 2- and 6-rowed barley isolines

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Abstract. The paramount importance of accumulated biomass in active-growing spikes over the number of grains per unit area has been well documented. However, it is not clear how different nitrogen (N) and radiation supplies during the active spike-growth phase alter the dynamics of floret primordia initiation and survival to establish the number of fertile florets and grains in 2- and 6-rowed barley. The objective of this paper was to evaluate how biomass and N partitioned between vegetative and reproductive organs alter the development of potential grains (i.e. floret primordia), when 2- and 6-rowed barley is grown under different radiation and N levels during their active spike-growth phase.

A field experiment was carried out using two near-isogenic lines differing in the spike type and grown under contrasting radiation and N levels around the active spike-growth phase. Floret primordia development and biomass and N partitioning towards vegetative and reproductive organs were analysed.

The results showed significant genotype × radiation × N level interactions on the dynamics of generation and abortion of reproductive structures. Under non-limiting N conditions, reductions in radiation levels strongly reduced the number of differentiated florets, although the effects were higher in 6- than in 2-rowed barley types. The higher the N supply, the higher the floret development stage reached when the spikes started growing at their maximum growth rates, increasing floret survival in that way. A threshold of floral development could not be found at any time in the crop cycle that guaranteed a fertile floret stage at heading. As it was not possible to identify a direct effect of N on the establishment of fertile florets, the efforts for further rising yield potential in barley should be focused on processes influencing partitioning of assimilates to reproductive growth during the critical period.

Additional keywords: biomass partitioning, fertile florets, malting barley, nitrogen, radiation.

Introduction

The critical period for yield determination in barley is set around the phases immediately before heading (HD) (Arisnabarreta and Miralles 2008*a*). The amount of assimilates partitioned towards the growing spikes during the critical period improves the establishment of fertile florets (FF) and grains in wheat (Stockman *et al*. 1983; Slafer *et al*. 1990; Fischer 1993; Abbate *et al*. 1995; Miralles *et al*. 1998; Demotes-Mainard *et al*. 1999; González *et al*. 2003) and barley (Prystupa *et al*. 2004; Arisnabarreta and Miralles 2008*b*). Although those approaches were achieved by altering incident radiation (Stockman *et al*. 1983; Fischer 1985; Abbate *et al*. 1995, 1997; Arisnabarreta and Miralles 2008*b*), mineral nutrition [e.g. nitrogen (N), phosphorus (P)] (Fischer 1993; Abbate *et al*. 1995; Demotes-Mainard *et al*. 1999, Demotes-Mainard and Jeuffroy 2001, 2004; Prystupa *et al*. 2004), photoperiod (Miralles *et al*. 2000; González *et al*. 2003, 2005) or genetics (Slafer *et al*. 1990; Slafer and Andrade 1991, 1993; Miralles *et al*. 1998), few contrasting reports in wheat analysed the interaction between N and radiation on the establishment of grain numbers (Abbate *et al*. 1995; Demotes-Mainard *et al*. 1999). While Demotes-Mainard *et al*. (1999) stated that the number of grains per spike is better predicted by changes in spike dry weight (SDW) at anthesis, Abbate *et al*. (1995) observed that spike N content at anthesis better predicted the number of grains per unit area (NGA) than SDW. In spite of the fact that these studies analysed the dynamic of biomass and N partitioning towards different organs within the plant, they only considered the final result on some yield components (i.e. grains per spike and/or grains per m^2), ignoring the intermediate processes that determine those components (e.g. floret primordia development per spike).

In that way, Arisnabarreta and Miralles (2008*b*) differentially decreased floret primordia development in 2- and 6-rowed barley

 10.1071/CP09292 1836-0947/10/070578

when shaded during the active spike-growth phase [i.e. between ~20 days pre-HD and HD]. In wheat different evidence (Langer and Hanif 1973; Whingwiri and Kemp 1980; Whingwiri and Stern 1982) has shown increases in primordia development when a higher amount of N was supplied, probably due to an increased partitioned biomass towards the spikes during the active spike-growth phase when N supply increased to the crop (Demotes-Mainard *et al*. 1999; Demotes-Mainard and Jeuffroy 2001, 2004). Although, as was described above, a large amount of evidence in the literature support that the process of development or survival of floret primordia is linked to spike growth, this approach has been recently questioned (Bancal 2008, 2009).

We are not aware of previous reports in the literature analysing the interaction between different radiation and N levels during the active spike-growth phase on biomass partitioning and its impact on the survival of reproductive structures in barley genotypes of different spike structure. We hypothesised that if a higher amount of N increase assimilates partitioning to the growing spikes, radiation restrictions during the active spike-growth phase will have a small impact when N is not limiting.

This work aims at understanding how biomass and N partitioned between vegetative and reproductive organs alter the development of potential grains (i.e. floret primordia), when 2- and 6-rowed barley are grown under different radiation and N levels during their active spike-growth phase.

Materials and methods

General conditions and experimental design

A field experiment was carried out in micro plots in large cylindrical containers (0.9 m height, 0.57 m diameter) during the 2003 growing season at the experimental field of the Department of Plant Production, University of Buenos Aires (34°35′S, 58°29′W) (for weather conditions see Arisnabarreta and Miralles 2008*a*). The experiment consisted of a factorial combination of two N fertiliser rates, two solar radiation levels and two near-isogenic barley lines (NIL). The NIL used (106 2R – 2-rowed – *Vrs1*, and 106 6R – 6-rowed – *vrs1*) were chosen because they had shown different floret primordia dynamics within the spike (Arisnabarreta and Miralles 2006, 2008*b*) and are homozygous in over 99% of the loci, unlike the lateral spikelet fertility gene (*Vrs1*). Pedigree of these genotypes is DAMPIER//(A14)PRIOR/YMER/3/KRISTINA (70S20–20)/4/ARITMONT. Seeds were kindly provided by Dr R. A. Richards, CSIRO, Australia.

The genotypes were hand-sown at 880 seeds/m^2 on 10 July 2003. High plant densities were used to guarantee the number of samples during the crop cycle and reduce the variability in the number of tillers produced between 2- and 6-rowed types. A week after seedling emergence, plots were thinned to a final density of 740 seedlings/m^2. Micro plots were sown in 7 rows and 0.09 m apart. In order to ensure low N availability to the crop, the containers were filled with a sand : soil mixture (3 : 1 by volume; 0.9 m depth). The soil used to prepare the sand : soil mixture was a silty clay loam soil classified as a Vertic Argiudoll according to USDA taxonomy. The bottom of each container was perforated and 0.1 m of gravel was added in order to improve water drainage.

One week before sowing, soil samples were extracted to determine N and P concentration. N content at that moment was 24 kg N/ha in the top 0.60-m soil layer. The P concentration available at sowing was 4 mg P per kg of soil (Bray and Kurtz 1945) in the top 0.20-m soil layer, and therefore 16 mg P per kg of soil was added as triple superphosphate before sowing.

Two radiation levels (unshaded control and shaded) were imposed during the active floret primordia mortality phase [i.e. from the maximum number of floret primordia (MNP) to HD stage] (~20 days' duration) (Arisnabarreta and Miralles 2006). The shaded treatment was achieved installing black plastic nets 0.1 m above the canopy, reducing 68 ± 0.5% of the incoming incident radiation measured at midday. N treatments consisted of a control-not fertilised (N_{24}; 24 kg N/ha available at sowing), and a fertilised treatment with ammonium nitrate (N_{234}; 24 kg N/ha available at sowing + 210 kg N/ha added throughout the crop cycle). To impose N treatments, two nutritional solutions containing macro- and micronutrients were prepared and mixed with the water used in the irrigation. The first nutritional solution containing SO_4K_2 6.4 mM as macronutrient, and Cl_2Mg 867 μM, $ZnCl_2.4H_2O$ 228 μM, $MnCl_2.4H_2O$ 664 μM, $CuCl_2.4H_2O$ 86 μM, H_3BO_3 118 μM, $CoCl_2.6H_2O$ 12 μM, $MoO_4Na_2.2H_2O$ 9 μM and $FeCl_3.6H_2O$ 1547 μM as micronutrients was used to impose the N_{24} treatment. The second nutritional solution was made with the same macro- and micronutrient concentrations used in the first solution, plus NO_3NH_4 2.6 mM as macronutrient, and it was used to impose the N_{234} treatment (Robson *et al*. 1995). Each container was irrigated with the corresponding solution every 3 days from sowing until the beginning of stem elongation, and every 2 days from this stage until maturity. Fungicides and insecticides were applied to prevent diseases and pests, and weeds were manually removed throughout the growing season.

Treatments were arranged in a completely randomised design with three replicates per treatment. ANOVA were performed by general linear model procedures to determine the treatment effects on crop traits. Significance among means were compared using the Least Significance Differences test (l.s.d., $\alpha = 0.05$). Relationships between variables were made by linear and bi-linear regressions (Anon. 1991).

Measurements, variables and analyses

From emergence, three plants per replicate from inner rows were randomly selected and apical development was observed by dissection of the main shoot apex every 2 days to determine their stages of development. After double ridge, the number of spikelet primordia initiated in the apex was counted to describe the dynamics of potential floret primordia initiation (RPI) and mortality (RPM), and the MNP initiated in the spike. The MNP stage was assumed when awn primordia appeared in the most developed spikelets within the apex (García del Moral *et al*. 1991). After the MNP stage was reached, floret primordia were considered alive when they maintained their green and turgid anthers. The rates of RPI and RPM (florets per degree-days) were estimated by linear regressions during the linear phase of RPI and RPM (Anon. 1991).

The developmental stages attained by florets on spikelets located in different positions within the spike were determined following the scale developed by Waddington *et al*. (1983).

Spikelets at different positions within the spike were dissected on the same side of the spike from the base (i.e. spikelet 2) to the apex (i.e. spikelet 20) in 3-spikelet intervals. In the 6-rowed lines, dissections were performed in the same positions, considering central (CSp) and lateral (LSp) spikelets (for details see fig. 1 in Arisnabarreta and Miralles 2006). Dynamics of floret development within each spikelet position were used to obtain the rate (RFD) and duration (DFD) of floret primordia development. DFD was measured as the difference between the thermal time when the maximum degree of floral development (W_{max}) was reached and the thermal time when all floral structures were clearly visible (W_4 pistil primodium present, Waddington *et al*. 1983). RFD was estimated as the inverse of DFD (i.e. $RFD = DFD^{-1}$).

Three main stem spikes per replicate from inner rows were sampled at HD (i.e. when 50% of the plants in each plot exposed the total spike over the flag leaf – code 58; Zadoks *et al*. 1974) to determine the number of FF per spike. A floret was considered fertile when style and stigmatic branches were spread, green anthers were visible, or pollen grains were set in well developed stigmatic branches, i.e. score 9.5–10 following the scale developed by Waddington *et al*. (1983). Floret survival was estimated as the relationship between the number of FF and MNP differentiated per spike in main shoots.

Dynamics of dry matter accumulation between MNP and HD [accumulated biomass (AGB)] in vegetative and reproductive organs were established weighing the main shoot stems and the spikes separately after drying them for 7 days at 70°C. Dynamics of dry matter accumulation in stems and spikes were fitted with a bi-linear model (González *et al*. 2003). The bi-linear model used was:

$$Y = a + bx\,(x \le c) + bc\,(x > c) + d\,(x - c)(x > c) \qquad (1)$$

Briefly, the model considers two phases, giving two distinct slopes. An initial slow rate phase of stem and spike growth, and a second fast growth rate phase until the final weight was reached. The parameters were *b*, the minimum growth rate of the organ, *c*, the timing of the inflection point (IP) when the spike and the stem began to grow at maximum rate, and *d*, the maximum growth rate of the organ (GR_{max}). As parameter *b* was not different from zero in all treatments, it is not shown in the results. Another parameter derived from the model is the duration of the phase of maximum growth rate (DGR_{max}), which was calculated by subtracting the parameter *c* from time to HD. The parameters of the fitted curves were compared by confidence intervals (95%). At HD, in the same samples taken to determine the number of FF, spike (SDW) and stems dry weight were determined weighting separately spikes and stems (including sheaths) after drying them at 70°C until constant weight.

Air temperature was measured (*i*) hourly in the field at 1.5 m using a meteorological station (Davis, Weather Monitor II, Hayward, CA, USA), and (*ii*) at the level of shoot apex from MNP to HD stage (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 less than 1°C. Thermal time from emergence was calculated using 0°C as the base temperature (Kirby 1988).

From emergence (Em) until HD, incident and transmitted PAR (photosynthetically active radiation) were measured at noon ± 1 h on clear days with a linear radiometer (LI-191 S, LI-COR Inc., Lincoln, NE, USA). PAR was estimated from total incident radiation using a conversion factor of 0.48 (Szeicz 1974).

The percentage of IR (IR%) was calculated as:

$$IR\% = 100 \times (1 - I/I_0) \qquad (2)$$

where I is the transmitted radiation measured above the senesced leaf layer, and I_0 is the incident radiation measured above the crop canopy.

Total incident radiation ($MJ/m^2.day$) was measured hourly at a standard meteorological station 200 m from the experimental site. A sigmoid function (Anon. 1991) was used to fit the dynamics of canopy IR% for each treatment. The intercepted cumulative radiation (PAR_{int}) was estimated by adding the product of daily intercepted radiation and daily total incident radiation.

Radiation use efficiency (RUE, g/MJ) during the MNP and HD phase was estimated by dividing the AGB and the PAR_{int} during this phase.

At harvest, plant samples from 0.3-m inner rows were taken, dried until constant weight, and weighed in order to estimate grain yield.

Results

Physiological yield components

Shading and N treatments significantly affected yield and AGB during the MNP–HD phase. In both barley types, yield variations (which ranged from 2 to 7 t/ha) were explained by changes in the NGA ($yield_{2\text{-row}} = 0.5$ NGA $- 0.9$, $R^2 = 0.99$, $P < 0.01$; $yield_{6\text{-row}} = 0.3$NGA $- 0.2$, $R^2 = 0.99$, $P < 0.001$). In the same way, the variations in the NGA were explained by changes in the number of FF per unit area ($NGA_{2\text{-row}} = 1.1$ FF per $m^2 - 1.1$, $R^2 = 0.98$, $P < 0.01$; $NGA_{6\text{-row}} = 0.7$ FF per $m^2 - 0.6$, $R^2 = 0.96$, $P < 0.05$). Averaging across treatments, the 2-row barley type produced significantly less AGB (187 g/m^2) than the 6-row type (304 g/m^2). The average AGB ($g/m^2 \pm$ s.e.) was 144.9 ± 40.7 and 345.7 ± 91.1 for the N_{24} and N_{234} treatments, respectively, while it was 338.5 ± 97.2 and 152.1 ± 37.8 for the control and shaded treatments, respectively (Table 1). Barley types did not significantly affect the PAR_{int}, although they affected the RUE (1.8 g/MJ and 3 g/MJ in 2- and 6-rowed types, respectively). The N treatments affected the PAR_{int} and RUE during the active phase of crop growth. Thus, the average PAR_{int} ($MJ/m^2 \pm$ s.e.) was significantly lower in the N_{24} (82.0 ± 14.9) than in the N_{234} (112.4 ± 21.6) treatment, while the average RUE (g/MJ ± s.e.) was 1.8 ± 0.3 and 3.0 ± 0.4 for the N_{24} and the N_{234} treatments, respectively. As expected, the PAR_{int} was significantly higher in the control (128.6 ± 12.4) than in the shaded treatment (65.8 ± 5.5), while the RUE was similar in both radiation regimes, i.e. 2.5 ± 0.6 and 2.2 ± 0.4 for the control and shaded treatment, respectively (Table 1).

Dynamics of reproductive primordia development

Independently of barley types and N treatments, spikelet primordia initiation began ~200 degree-days after emergence (i.e. collar stage) (Fig. 1). The 6-rowed barley produced a higher MNP per spike (116) than the 2-rowed (41) barley, due to their

Table 1. Grain yield (kg/ha), accumulated above-ground biomass (AGB; g/m^2), accumulated photosynthetically active radiation intercepted (PAR$_{int}$; MJ/m^2) and radiation use efficiency (RUE; g/MJ) between the maximum number of floret primordia stage and heading stage in the 2- and 6-rowed near-isogenic barley lines, shading and nitrogen (N) treatments

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

NIL	Radiation	N	Yield	AGB	PAR$_{int}$	RUE
2-rowed	C	N_{24}	3351	127	103	1.3
		N_{234}	7560	380	153	2.5
	S	N_{24}	2155	69	57	1.2
		N_{234}	5897	169	72	2.3
6-rowed	C	N_{24}	2691	261	112	2.4
		N_{234}	6869	586	146	4.0
	S	N_{24}	2195	122	56	2.2
		N_{234}	5439	248	78	3.2
NIL (G)		–	n.s.	***(87)	n.s.	**(0.8)
Radiation (S)		–	**(764)	***	***	n.s.
Nitrogen (N)		–	***(764)	*	***	**(0.8)
G × S		–	n.s.	n.s.	n.s.	n.s.
G × N		–	n.s.	n.s.	n.s.	n.s.
S × N		–	n.s.	*(123)	*(15)	n.s.
G × S × N		–	n.s.	n.s.	n.s.	n.s.

higher RPI rate ($P < 0.001$). Both barley types did not differ in the duration of primordia initiation ($P > 0.05$) (Fig. 1, Table 2).

A significant G × N interaction was observed in the MNP differentiated per spike, due to the fact that higher N supply treatment increased the MNP more in 6- than in 2-rowed genotypes (Fig. 1, Table 2). The differences in the MNP observed between N treatments were explained by slight changes in RPI and in the duration of the Em–MNP phase (Table 2).

N supply affected floret development as early as the MNP stage (Fig. 2). With the exception of LSp, the floral score at the MNP stage in all spikelet positions within the spike was lower under the N_{24} treatment (Fig. 2).

Independently of the treatments, RPM began at 800 degree-days from emergence. However, N treatments started to clearly affect RPM from 1000 degree-days onwards (Fig. 1). The significant interaction between barley types and shading ($P < 0.05$) for the RPM revealed that shading applied during the MNP–HD phase affected the RPM more in 6- than in 2-rowed genotypes (Table 2). However, the significant S × N interaction ($P < 0.05$) indicates that the shaded treatment affected the RPM more in the N_{234} than in the N_{24} treatment (Table 2).

Florets primordia initiated their development in all positions within the spike and continued until different degrees of

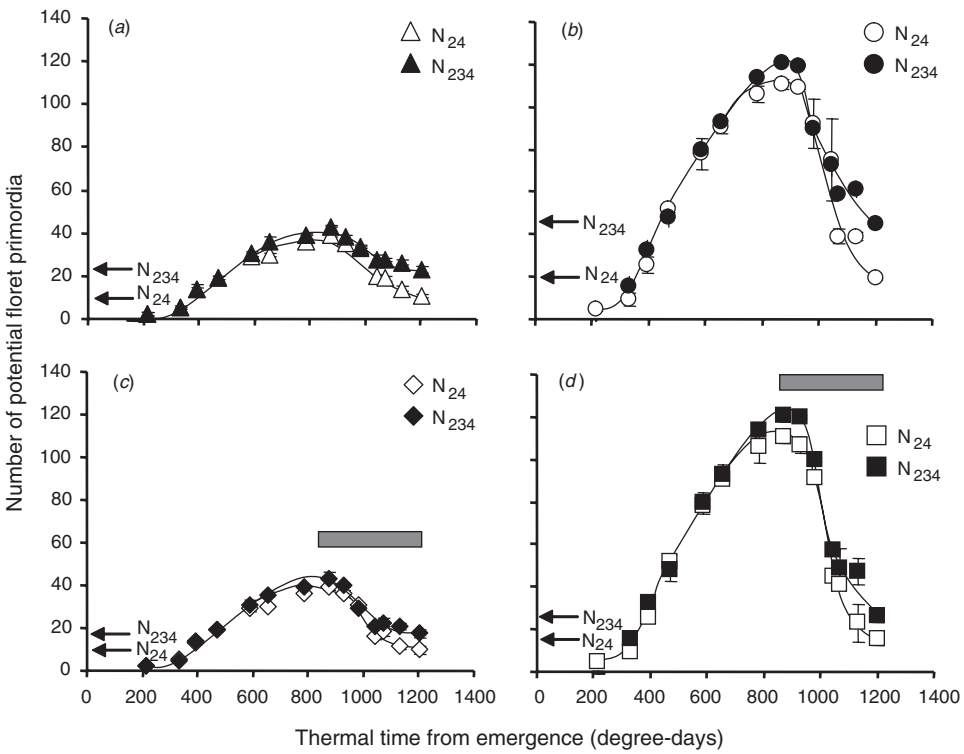

Fig. 1. Dynamics of floret primordia initiation and mortality against thermal time from emergence for the (*a*, *c*) 2- and (*b*, *d*) 6-rowed near-isogenic lines. Panels *a*, *b*, and *c*, *d* refer to the control and shaded treatments, respectively. Open and closed symbols represent the low (N_{24}) and the high (N_{234}) nitrogen supply treatments, respectively. Vertical lines are the standard errors. Horizontal bars indicate the period of time when shading treatments were applied. Arrows indicate the number of fertile florets at heading for each treatment. Curves were fitted by hand.

Table 2. Rate (florets per degree-days × 10^{-3}) of floret primordia initiation (RPI) and mortality (RPM), maximum number of floret primordia differentiated per spike (MNP), fertile florets per spike (FF), floret primordia survival [100*(FF MNP^{-1})], and thermal time from emergence (Em) to MNP and heading (HD), in the 2- and 6-rowed near-isogenic barley lines, shading and nitrogen (N) treatments

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

Genotypes	Shading	N	RPI	RPM	MNP	FF	Survival (%)	Em–MNP	Em–HD
2-row	C	N_{24}	62	96	39	11	27	786	1201
		N_{234}	68	61	43	23	53	811	1179
	S	N_{24}	62	101	39	10	25	777	1201
		N_{234}	68	82	43	18	41	799	1179
6-row	C	N_{24}	181	328	111	19	17	789	1186
		N_{234}	192	253	121	45	37	813	1179
	S	N_{24}	185	340	111	15	14	779	1193
		N_{234}	199	328	121	27	22	796	1186
NIL (G)		–	***(10)	***	***	***	***	n.s.	n.s.
Shading (S)		–	n.s.	***	n.s.	***	***	*(12)	n.s.
Nitrogen (N)		–	n.s.	***	***	***	***	**(12)	***
G × S		–	n.s.	*(20)	n.s.	**	n.s.	n.s.	n.s.
G × N		–	n.s.	n.s.	**(3)	***	*(4)	n.s.	*(9)
S × N		–	n.s.	*(20)	n.s.	***	***(4)	n.s.	n.s.
G × S × N		–	n.s.	n.s.	n.s.	*(4)	n.s.	n.s.	n.s.

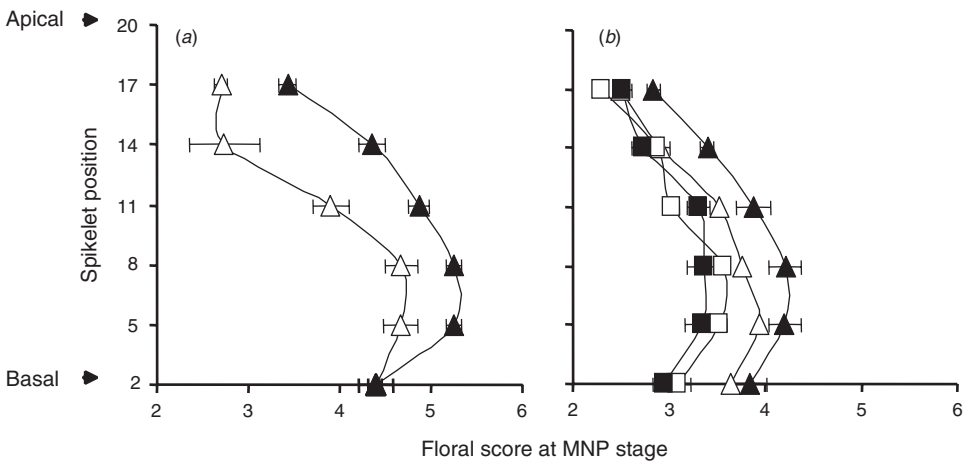

Fig. 2. Floral score at maximum number of floret primordia stage in different spikelet positions within the spike in the (*a*) 2- and (*b*) 6-rowed lines. Symbols correspond to central spikelets in 2- and 6-rowed (△▲), and lateral spikelets in 6-rowed barley (□ ■). Open and closed symbols correspond to the low (N_{24}) and high (N_{234}) nitrogen treatments, respectively. Horizontal lines indicate standard errors.

development were reached (Figs 3, 4). In the 2-rowed type, florets continued developing in distal positions (e.g. spikelet 14) in contrast to the 6-rowed barley (Figs 3, 4). In addition, in the 6-rowed type, a higher number of LSp stopped their floral development earlier in relation to those positioned in CSp (e.g. spikelets 2, 14) (data not shown).

A significant interaction between shading and N treatments was observed in the dynamics of floret development (Figs 3, 4). While in the N_{24} treatment radiation did not change the dynamics of floral development, when plants were grown at the N_{234} treatment shading reduced floral development in distal spikelet positions (Figs 3, 4). However, a high N supply hastened floret development in both barley types in distal spikelets, as well as in CSp as in LSp positions (e.g. spikelets 2, 11) (Figs 3, 4).

The N and radiation treatments affected the dynamics of floret development, altering the maximum stage of floral development (W_{max}) attained within each spikelet position. The N_{234} treatment increased W_{max} 24 and 30% in 2- and 6-rowed types, respectively. In 6-rowed barley, the higher N supply treatment increased maximum floral score by 25 and 35% in CSp and LSp, respectively (Figs 3, 4). On the other hand, the shaded treatment only reduced W_{max} in the N_{234} but not in the N_{24} treatment (Figs 3, 4).

The higher stage of floral development reached in the N_{234} treatment, with respect to the low N supply, seemed to be more associated with changes in the RFD, and to a lesser extent to the DFD. In fact, the N_{234} treatment increased the RFD and DFD 50 and 20%, respectively. However, the RFD and DFD were only slightly (<10%) reduced by shading treatments (Figs 3, 4).

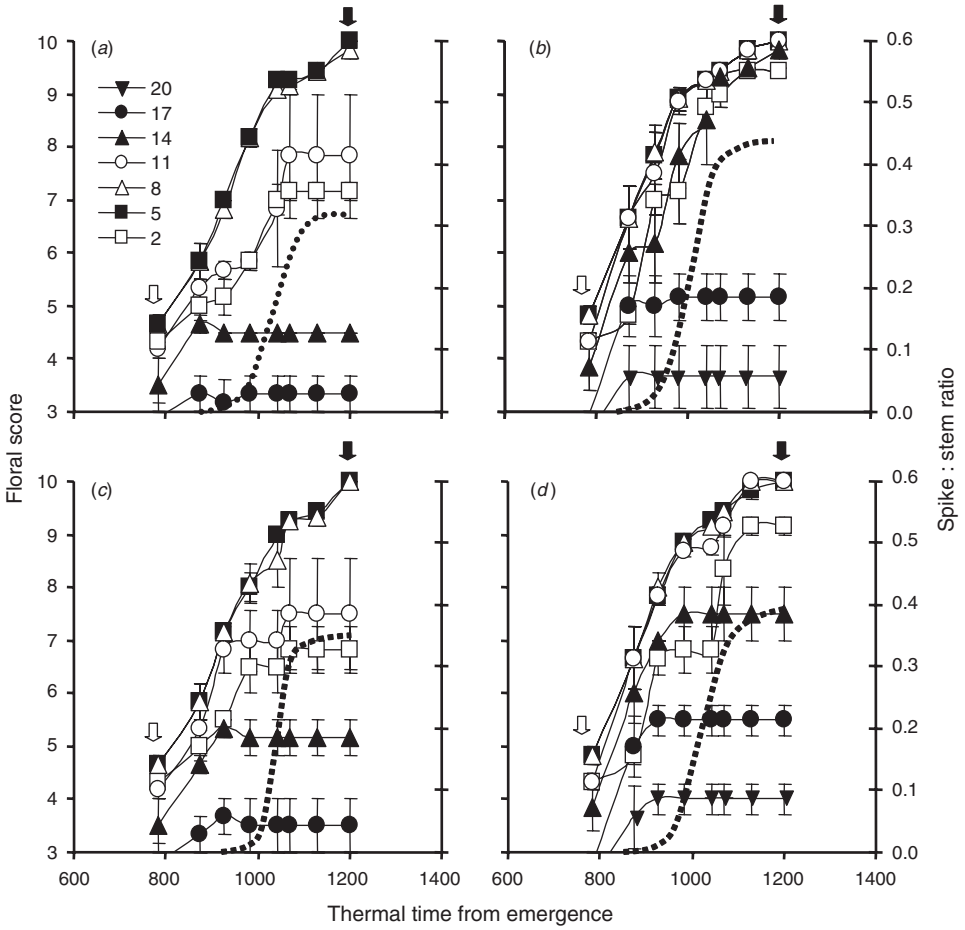

Fig. 3. Dynamics of floral development in different spikelet positions (i.e. 2, 5, 8, 11, 14, 17 and 20, the most apical) in the 2-rowed barley in the low (N_{24}) (*a*, *c*) and high (N_{234}) (*b*, *d*) nitrogen treatments. Panels *a*, *b*, and *c*, *d* correspond to the control and shaded treatments, respectively. Vertical lines indicate standard errors. Open and closed arrows indicate the maximum number of floret primordia and heading stages, respectively. Dotted lines are the fitted curves for the spike : stem ratio.

The final number of FF per spike and floret survival was significantly affected by G, S and N treatments. Although in all cases the number of FF was ~2-fold higher in the N_{234} (28) compared with the N_{24} treatment (14), the significant G × S × N interaction ($P < 0.05$) indicated that the negative effect of shading on FF was higher when applied in the 6- rather than in the 2-rowed barley type at the high N fertilisation rate. In fact, the reduction of FF in the N_{234} treatment when shading was applied, respect to the control (unshaded) was 22 and 40% in the 2- and 6-rowed type, respectively (Table 2). The significant interaction found between shading and N ($P < 0.001$) indicates that shading affected floret survival only when the crop was fertilised with the higher N fertilisation rate (Table 2).

Nitrogen and biomass partitioning between vegetative and reproductive organs

Spikes began growth at their maximum rates (IP_{spike}) ~170 degree-days later than the stems (IP_{stem}) (Table 3). The maximum spike and stem growth rate was overlapped between 1000 and 1200 degree-days after emergence, coinciding with the period of time when differences in floret RPM were magnified between treatments (Fig. 1).

The lower N supply treatment tended to delay (*i*) HD and (*ii*) the beginning of IP_{spike} and IP_{stem}. Regarding HD, the difference between both N rates, although significant, was irrelevant in agronomical terms (~14 degree-days). In the same way, the differences in IP_{spike} and IP_{stem} between both N treatments were also negligible (~20 degree-days) (Tables 2, 3). On the other hand, the shaded treatments did not modify the IP_{spike}, IP_{stem} and HD time, and consequently DGR_{max} of both organs was the same in both treatments (Table 3).

As expected, the N_{234} treatment increased GR_{max} of stems and spikes. The N_{234} treatment increased GR_{max} of spikes and stems 150 and 100%, respectively (Table 3). Although the shaded treatment reduced GR_{max} of stems and spikes, the impact depended on whether plants were fertilised or not (Table 3). The spikes and stems GR_{max} were more affected by shading when plants were grown under the N_{234} treatment demonstrating an S × N interaction (Table 3).

Spike : stem ratio sharply increased from ~200 degree-days before HD, i.e. in the middle of the MNP–HD phase,

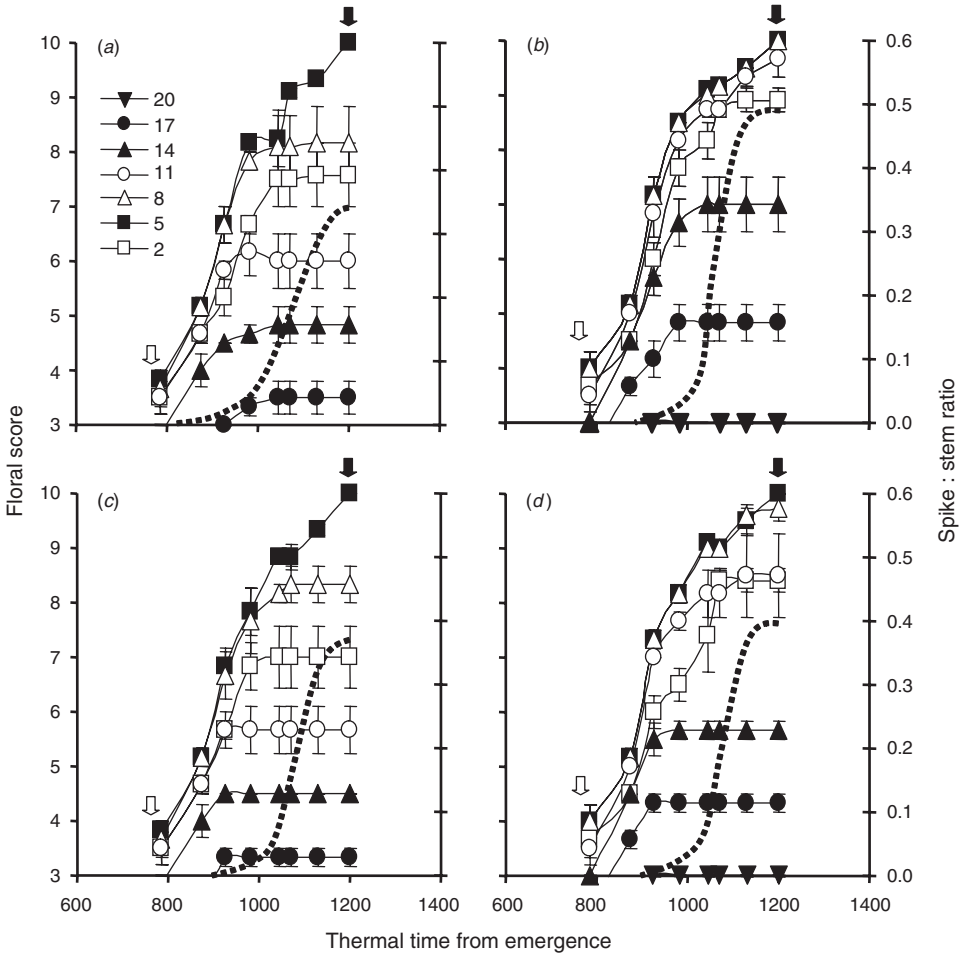

Fig. 4. Dynamics of floral development in different spikelet positions (i.e. 2, 5, 8, 11, 14, 17 and 20, the most apical) in the 6-rowed barley in central spikelet positions in the (*a*, *c*) low (N_{24}) and (*b*, *d*) high (N_{234}) nitrogen treatments. Panels *a*, *b*, and *c*, *d* correspond to the control and shaded treatments, respectively. Vertical lines indicate standard errors. Open and closed arrows indicate the maximum number of floret primordia and heading stages, respectively. Dotted lines are the fitted curves for the spike : stem ratio.

Table 3. Parameters derived from a bi-linear model describing spike and stem growth for the different treatments

The parameters are: the thermal time from emergence when the organ began to grow at maximum rate (IP ± s.e.), the maximum growth rate of the organ (GR_{max} ± s.e.) (g per degree-days × 10^{-4}) and the duration of the phase of maximum growth rate (DGR_{max} ± s.e.) (degree-days)

Spike type	Shading	N	Spike			Stem		
			IP_{spike}	GR_{max}	DGR_{max}	IP_{stem}	GR_{max}	DGR_{max}
2-row	C	N_{24}	968 ± 12	3.6 ± 0.2	233	856 ± 27	6.9 ± 0.6	345
		N_{234}	942 ± 29	11.8 ± 1.6	259	831 ± 19	17.2 ± 1.1	370
	S	N_{24}	964 ± 30	2.5 ± 0.4	237	786 ± 55	3.7 ± 0.6	415
		N_{234}	969 ± 30	6.4 ± 0.9	232	746 ± 57	7.5 ± 1.0	455
6-row	C	N_{24}	1024 ± 9	6.4 ± 0.4	177	858 ± 30	8.3 ± 0.8	343
		N_{234}	1019 ± 18	15.8 ± 1.8	182	854 ± 17	15.5 ± 0.9	347
	S	N_{24}	1049 ± 5	5.0 ± 0.2	152	821 ± 44	4.5 ± 0.6	380
		N_{234}	1016 ± 38	8.3 ± 2.1	185	815 ± 37	8.5 ± 1.0	386

when floret primordia in distal positions within the spike interrupted their development (Figs 3, 4). A higher amount of assimilates partitioned to the spikes during their active growth phase resulted in a higher proportion of spikelets reaching the fertile floral stage (see treatment N_{234}, control; Fig. 3). In addition, the N_{234} treatment significantly increased spike : stem ratio between 1000 and 1200 degree-days from emergence (Figs 3, 4).

The number of FF per spike was positively correlated with SDW at HD in both genotypes (Fig. 5*a*). Although the ordinates in

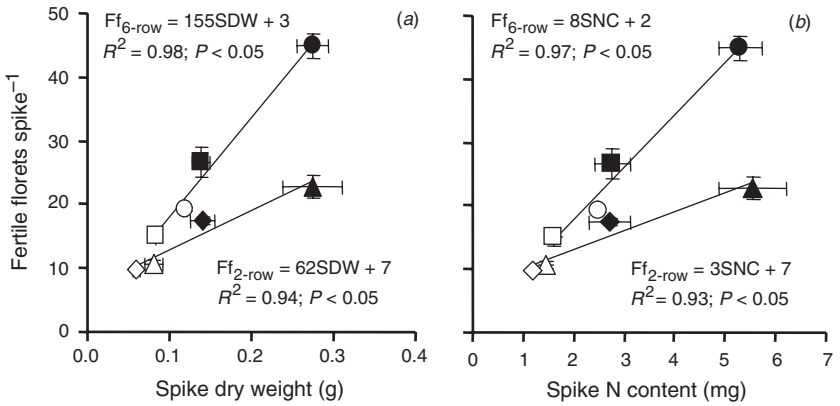

Fig. 5. Relationships between fertile florets per spike and (*a*) spike dry weight, and (*b*) spike nitrogen content at heading in 2- (△▲◇◆) and 6-rowed barley (○●□■) for the control (△▲○●) and shaded (◇◆□■) treatments in the low (N_{24}) (open symbols) and high (N_{234}) (closed symbols) nitrogen treatments. Regressions were fitted considering the 2- and 6-rowed barley separately. Vertical and horizontal lines are standard errors.

both adjustments did not differ from zero ($P > 0.05$), the 2- and 6-rowed types differed ($P < 0.01$) in their slopes, i.e. 0.06 and 0.16 florets/mg of SDW, respectively. When the FF was plotted against the spike N content at HD, the fitted regression, as well as the distribution of residuals did not improve compared with the relationship between FF per spike and SDW at HD (Fig. 5*b*).

Discussion

The present work highlights the significant genotype × radiation level × N supply interaction over the generation and abortion of reproductive structures that determine grain yield potential in barley. N shortage slightly reduced the MNP, supporting the fact that in barley, as well as in many other species, this trait is under genetic control (Arisnabarreta and Miralles 2004, 2008*b*). As was described in wheat (Langer and Hanif 1973; Whingwiri and Kemp 1980; Whingwiri and Stern 1982; Sibony and Pinthus 1988; Miralles *et al*. 2000; Li *et al*. 2001; González *et al*. 2003), the main effects of different nutritional and radiation environments during the active spike-growth phase in barley operated on the floret primordia survival. In addition, the floret primordia of LSp in the 6-rowed type were more susceptible to degeneration, failing to reach a FF stage under stressful N and radiation conditions. LSp are probably weaker sinks with respect to central ones, and thereby more susceptible when the source is reduced.

As was previously observed in wheat (Stockman *et al*. 1983; Kirby 1988; González *et al*. 2003), and barley (Arisnabarreta and Miralles 2006), the floret mortality phase was overlapped with the stem and the spike active-growing phase, suggesting a high assimilate competition between reproductive and vegetative organs when the fate of a floret primordia is being defined. Thus, a high assimilate partitioning during the active spike-growth phase determines the successful establishment of FF and grain yield. However, other evidence suggests that the fertility of the floret primordia could be modified as a consequence of 'direct' effects of N when this nutrient is applied (Abbate *et al*. 1995; Sinclair and Jamieson 2006).

In agreement with Demotes-Mainard *et al*. (1999), in the present study a higher N rate increased the biomass partitioned to the spikes. Additionally, the relationship found between the number of FF and the spike N content did not improve the relationship between FF and SDW, suggesting that N had not had a 'direct' effect on the fate of the floret primordia previously initiated. Thus, the results of the present study reinforce the hypothesis that carbohydrates partitioning in the spike is the driving force that determines whether a floret primordia successfully reaches the FF stage. Therefore, the effect of N on floret primordia appears to be more an 'indirect' (consequence) than a 'direct' (cause) mediated by changes in carbohydrate production. In that sense, the positive effect of N on FF per spike during the active spike-growth phase was associated with higher (*i*) PAR_{int}, (*ii*) RUE, and (*iii*) assimilates allocation to reproductive organs. These results reinforce the hypothesis that further breeding efforts for increasing yield potential in wheat and barley must be concentrated on increasing spike fertility and RUE during the phases immediately before HD (Reynolds *et al*. 2009).

Taking into account the N supply increased the floret development and FF, the immediate question is: which is the non-return degree of floret development to successfully reach the stage of FF? Previous studies in wheat, observed that floret death started when the most proximal floret to the rachis into the spikelet reached the stage W_8 (stigmatic branches elongating) (Craufurd and Cartwright 1989), or between W_7 and W_8 (styles elongating) (Sibony and Pinthus 1988), suggesting that floret death might be related to a developmental process and not to a shortage of assimilates to the spike growth. In agreement with Bancal (2009), in the present work the high N supply treatment increased floret development in all spikelet positions, however, it was not possible to identify the threshold of floral development proposed in the literature. In fact, a floret primordia was fertile at HD if it reached a floral score between W_3 and $W_{5.25}$ at the MNP stage (Fig. 6*a*), or between W_7 and W_9 when the last internodes began their elongation (Fig. 6*b*). When the probability of floret primordia to be established as fertile was plotted against the degree of development of the same primordia at the beginning of the stem elongation phase (Fig. 6*b* inset), a floret primordia that reached

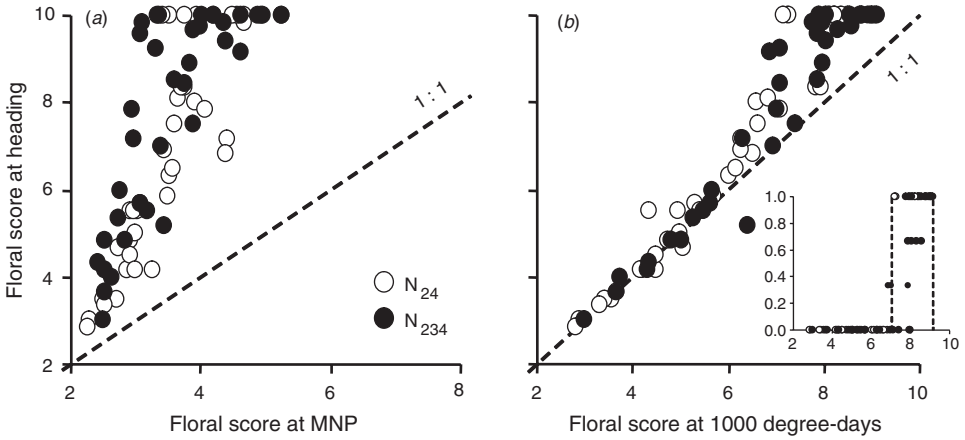

Fig. 6. Relationship between floral score (Waddington *et al*. 1983) attained at heading and floral score attained at (*a*) maximum number of floret primordia stage, and (*b*) 1000 degree-days after emergence, in different spikelet positions in the low (N_{24}) and high (N_{234}) nitrogen treatments. Inset: relationship between the probability of floret primordia reaching a fertile floret stage at heading and the floral score attained at 200 degree-days before heading.

a stage between W_7 and W_9 had a probability of 0, 33, 66 or 100% to be fertile. This wide range of floral development could be explained by differences in the rate of floral development between barley types, spikelet positions, N and shading treatments (Table 3).

This paper demonstrated that the coordination of the degree of floral development attained at MNP changed under contrasting nutritional conditions. Therefore, the higher degree of floret development at MNP under the higher N supply conditions might give a better chance to the FF to complete those primordia previously differentiated when the spikes started their active growth phase.

Initially, we hypothesised that if a higher amount of N increased assimilates partitioning to the growing spikes, radiation restrictions during the active spike-growth phase would have a small impact when N was not limiting. However, the results did not support this hypothesis, because radiation restrictions during the active spike-growth phase strongly affected, in absolute and in relative terms, the survival of floret primordia when N was non-limiting.

From this paper it is possible to conclude that the proportion of florets is linked to spike growth more than a direct effect of N into the spikes. In fact, under non-limiting N conditions, reductions in radiation levels, when the spike is growing at maximum rate, strongly reduced the definition of potential grains. N promoted a higher degree of floret development. However, a threshold of floral development could not be found at any time in the crop cycle that guaranteed a FF stage at HD. As it was not possible to identify a direct effect of N on the establishment of FF, the efforts of breeding for further rising yield potential in barley should be focused on processes and the genetic bases influencing partitioning of assimilates to reproductive growth during the critical period.

Acknowledgments

We thank L. Hercum for technical assistance in the experimental field and C. Hortis, F. G. González and E. M. Whitechurch for comments on the manuscript. This work was partially supported by the University of Buenos Aires (UBACyT AG-15, G023), CONICET (PIP 02415) and the International Foundation for Science (IFS 2804/1). During the experiments S. Arisnabarreta held a postgraduate scholarship from the University of Buenos Aires.

References

Abbate PE, Andrade FE, Culot JP (1995) The effects of radiation and nitrogen on number of grains in wheat. *Journal of Agricultural Science, Cambridge* **124**, 351–360. doi:10.1017/S0021859600073317

Abbate PE, Andrade FE, Culot JP, Bindraban PS (1997) Grain yield in wheat: effects of radiation during spike growth period. *Field Crops Research* **54**, 245–257. doi:10.1016/S0378-4290(97)00059-2

Anon. (1991) 'Table curve V. 3.0. User's manual. Version 3.0 AISN Software.' (Jandel Scientific: Corte Madera, CA)

Arisnabarreta S, Miralles DJ (2004) The influence of fertilizer nitrogen application on development and number of reproductive primordia in field grown two- and six-rowed barleys. *Australian Journal of Agricultural Research* **55**, 357–366. doi:10.1071/AR03066

Arisnabarreta S, Miralles DJ (2006) Yield responsiveness in two- and six-rowed barley grown in contrasting nitrogen environments. *Journal of Agronomy & Crop Science* **192**, 178–185. doi:10.1111/j.1439-037X.2006.00203.x

Arisnabarreta S, Miralles DJ (2008*a*) Critical period for grain number establishment of near isogenic lines of two- and six-rowed barley. *Field Crops Research* **107**, 196–202. doi:10.1016/j.fcr.2008.02.009

Arisnabarreta S, Miralles DJ (2008*b*) Radiation effects on potential number of grains per spike and biomass partitioning in two- and six-rowed near isogenic barley lines. *Field Crops Research* **107**, 203–210. doi:10.1016/j.fcr.2008.01.005

Bancal P (2008) Positive contribution of stem growth to grain number per spike in wheat. *Field Crops Research* **105**, 27–39. doi:10.1016/j.fcr.2007.06.008

Bancal P (2009) Early development and enlargement of wheat floret primordia suggest a role of partitioning within spike to grain set. *Field Crops Research* **110**, 44–53. doi:10.1016/j.fcr.2008.06.014

Bray RH, Kurtz LT (1945) Determination of total, organic and available forms of phosphorus in soils. *Soil Science* **59**, 39–45. doi:10.1097/00010694-194501000-00006

Craufurd PQ, Cartwright PM (1989) Effect of photoperiod and chlormequat on apical development and growth in a spring wheat (*Triticum aestivum*) cultivar. *Annals of Botany* **63**, 515–525.

Demotes-Mainard S, Jeuffroy MH (2001) Partitioning of dry matter and nitrogen to the spike throughout the spike growth period in wheat crops subjected to nitrogen deficiency. *Field Crops Research* **70**, 153–165. doi:10.1016/S0378-4290(01)00133-2

Demotes-Mainard S, Jeuffroy MH (2004) Effects of nitrogen and radiation on dry matter and nitrogen accumulation in the spike of winter wheat. *Field Crops Research* **87**, 221–233. doi:10.1016/j.fcr.2003.11.014

Demotes-Mainard S, Jeuffroy MH, Robin S (1999) Spike dry matter and nitrogen accumulation before anthesis in wheat as affected by nitrogen fertilizer: relationship to kernels per spike. *Field Crops Research* **64**, 249–259. doi:10.1016/S0378-4290(99)00046-5

Fischer RA (1985) Number of kernels in wheat crops and the influence of solar radiation and temperature. *Journal of Agricultural Science, Cambridge* **105**, 447–461. doi:10.1017/S0021859600056495

Fischer RA (1993) Irrigated spring wheat and timing and amount of nitrogen fertilizer. II. Physiology of grain yield response. *Field Crops Research* **33**, 57–80. doi:10.1016/0378-4290(93)90094-4

García del Moral MB, Jimenez Tejada MP, García del Moral LF, Ramos JM, Roca de Togores F, Molina-Cano JL (1991) Apex and ear development in relation to the number of grains on the main-stem ears in spring barley (*Hordeum distichon*). *Journal of Agricultural Science, Cambridge* **117**, 39–45. doi:10.1017/S0021859600078941

González FG, Slafer GA, Miralles DJ (2003) Grain and floret number in response to photoperiod during stem elongation in fully and slightly vernalized wheats. *Field Crops Research* **81**, 17–27. doi:10.1016/S0378-4290(02)00195-8

González FG, Slafer GA, Miralles DJ (2005) Photoperiod during stem elongation in wheat: is its impact on fertile floret and grain number determination similar to that of radiation? *Functional Plant Biology* **32**, 181–188. doi:10.1071/FP04103

Kirby EJM (1988) Analysis of leaf, stem and ear growth in wheat from terminal spikelet stage to antesis. *Field Crops Research* **18**, 127–140. doi:10.1016/0378-4290(88)90004-4

Langer RHM, Hanif M (1973) A study of floret development in wheat (*Triticum aestivum* L.). *Annals of Botany* **37**, 743–751.

Li C, Cao W, Dai T (2001) Dynamic characteristics of floret primordium development in wheat. *Field Crops Research* **71**, 71–76. doi:10.1016/S0378-4290(01)00144-7

Miralles DJ, Katz SD, Colloca A, Slafer GA (1998) Floret development in near isogenic wheat lines differing in plant height. *Field Crops Research* **59**, 21–30. doi:10.1016/S0378-4290(98)00103-8

Miralles DJ, Richards RA, Slafer GA (2000) Duration of stem elongation period influences the number of fertile florets in wheat and barley. *Australian Journal of Plant Physiology* **27**, 931–940.

Prystupa P, Savin R, Slafer GA (2004) Grain number and its relationship with dry matter, N and P in the spikes at heading in response to N × P fertilization in barley. *Field Crops Research* **90**, 245–254. doi:10.1016/j.fcr.2004.03.001

Reynolds MP, Foulkes MJ, Slafer GA, Berry P, Parry MAJ, Snape JW, Angus WJ (2009) Raising yield potential in wheat. *Journal of Experimental Botany* **60**, 1899–1918. doi:10.1093/jxb/erp016

Robson AD, Osborne LD, Snowball K, Simmons WJ (1995) Assessing sulfur status in lupins and wheat. *Australian Journal of Experimental Agriculture* **35**, 79–86. doi:10.1071/EA9950079

Sibony M, Pinthus MJ (1988) Floret initiation and development in sprint wheat (*Triticum aestivum* L.). *Annals of Botany* **61**, 473–479.

Sinclair TR, Jamieson PD (2006) Grain number, wheat yield, and bottling beer: an analysis. *Field Crops Research* **98**, 60–67. doi:10.1016/j.fcr.2005.12.006

Slafer GA, Andrade FE (1991) Changes in physiological attributes of the dry matter economy of bread wheat (*Triticum aestivum* L.) through genetic improvement of grain yield potential at different regions of the world. A review. *Euphytica* **58**, 37–49. doi:10.1007/BF00035338

Slafer GA, Andrade FE, Feingold FE (1990) Genetic improvement of bread wheat (*Triticum aestivum* L.) in Argentina: relationships between nitrogen and dry matter. *Euphytica* **50**, 63–71. doi:10.1007/BF00023162

Slafer GA, Andrade FH (1993) Physiological attributes related to the generation of grain yield in bread wheat cultivars released at different eras. *Field Crops Research* **31**, 351–367. doi:10.1016/0378-4290(93)90073-V

Stockman YM, Fischer RA, Brittain EG (1983) Assimilate supply and floret development within the spike of wheat (*Triticum aestivum* L.). *Australian Journal of Plant Physiology* **10**, 585–594. doi:10.1071/PP9830585

Szeicz G (1974) Solar radiation for plant growth. *Journal of Applied Ecology* **11**, 617–636. doi:10.2307/2402214

Waddington SR, Cartwright PM, Wall PC (1983) A quantitative scale of spike initial and pistil development in barley and wheat. *Annals of Botany* **51**, 119–130.

Whingwiri EE, Kemp DR (1980) Spikelet development and grain yield of the wheat ear in response to applied nitrogen. *Australian Journal of Agricultural Research* **31**, 637–647. doi:10.1071/AR9800637

Whingwiri EE, Stern WR (1982) Floret survival in wheat: significance of the time of floret initiation relative to terminal spikelet formation. *Journal of Agricultural Science, Cambridge* **98**, 257–268. doi:10.1017/S0021859600041794

Zadoks JC, Chang TT, Konzak CF (1974) A decimal code for the growth stage of cereals. *Weed Research* **14**, 415–421. doi:10.1111/j.1365-3180.1974.tb01084.x

Manuscript received 13 October 2009, accepted 13 May 2010