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Wheat floret survival as related to pre-anthesis spike growth

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Abstract

Further improvements to wheat yield potential will be essential to meet future food demand. As yield is related to the number of fertile florets and grains, an understanding of the basis of their generation is instrumental to raising yield. Based on (i) a strong positive association between the number of fertile florets or grains and spike dry weight at anthesis; and (ii) the finding that floret death occurs when spikes grow at maximum rate, it was always assumed that floret survival depends on the growth of the spike. However, this assumption was recently questioned, suggesting that assimilates diverted to the spike do not determine the number of florets and grains and that the onset of floret death may instead be a developmental process that is not associated with spike growth. In this study, the relationships between the fate of floret primordia and spike growth from six independent experiments that included different growing conditions (greenhouse/field experiments, growing seasons, photoperiod/shading treatments during the floret primordia phase) and diverse cultivar types (winter/spring, semi-dwarf/standard-height, photoperiod sensitive/insensitive) were re-analysed together. Onset of floret death was associated with the beginning of spike growth at the maximum rate in *c.* 80% of the cases analysed; and the rate of floret death (the main determinant of floret survival) showed a negative quantitative relationship with spike weight at anthesis. As floret death and survival were shown to be linked to pre-anthesis spike growth, the strategy of focusing on traits associated with pre-anthesis spike growth when breeding to increase wheat yield potential further is valuable.

Key words: Beginning of spike growth, fertile florets, onset of floret death, yield potential.

Introduction

Production, yield potential, and food security

It has been estimated that global cereal production should increase during the first half of the 21st century by *c.* 50% in order to satisfy the expected demand (Rosegrant and Cline, 2003). At present, wheat accounts for *c.* 30% of global grain production and *c.* 45% of cereals used as food (directly or indirectly), being the major crop providing energy and protein for humankind (Chand, 2009). The actual rate of wheat production increase (0.54% per year between 1997 and 2007) is less than half of that required in the near future (1.32% annual increase). As the area cropped with wheat may only marginally increase, further production must be mainly achieved by increasing yield (Reynolds *et al.*, 2009).

Abbreviations: AN, anthesis; CGR, crop growth rate (g m^{-2} d^{-1}); DSG, duration of spike growth (d or °Cd); F1, floret primordium most proximal to the rachis within the central spikelet; FE, fruiting efficiency (grain number g^{-1} spike at anthesis); FF, number of fertile florets per unit area at anthesis; FI, floral initiation; Fn, floret primordium most distal from the rachis within the central spikelet; FPP, floret primordia phase, from terminal spikelet to anthesis; GN, grain number per unit area; GS, grain set; MaxNFP, maximum number of living floret primordia within a spikelet; MinNFP, minimum number of living floret primordia within a spikelet; NP+0, natural photoperiod; NP+3, extension of 3 h over the natural photoperiod; NP+6, extension of 6 h over the natural photoperiod; NP–3, reduction of 3 h from the natural photoperiod; RFD, rate of floret death; RNFP, relative number of floret primordia; SDW, spike dry weight at anthesis (g m^{-2}); SE, stem elongation phase or FPP; SF, survival of floret primordia; SPI, spike partitioning index; TS, terminal spikelet.

Improvements in yield potential (i.e. yield without hail, frost or lodging and without water, nutrient or biotic stress) have been a major contributor to increased crop yields in the past (Reynolds *et al.*, 2009; Fischer and Edmeades, 2010). This is because cultivars with higher yield potential generally express their advantage under a wide range of suboptimal environments as well (Fischer, 1984, 2007; Calderini and Slafer, 1998; Slafer and Araus, 2007; Reynolds *et al.*, 2009; Fischer and Edmeades, 2010).

However, raising yield potential further does not seem to be an easy task. The reduction in rate of gains in wheat production observed during recent decades is a consequence not only of a reduction in wheat area under cropping but also a generalized deceleration in wheat yield progress (Calderini and Slafer, 1998; Slafer and Peltonen-Saino, 2001; Joshi *et al.*, 2007; Zhou *et al.*, 2007; Chand, 2009; Peltonen-Saino *et al.*, 2009; Brisson *et al.*, 2010; Fischer and Edmeades, 2010). The reduced growth rate of wheat yield may reflect a reduced progress in yield potential of the cultivars (Fischer and Edmeades, 2010). The genetic gain in yield potential since the green revolution has been around 1% per year (Sayre *et al.*, 1997; Abbate *et al.*, 1998; Shearman *et al.*, 2005; Zhou *et al.*, 2007), which is lower than the predicted increase in global demand (Reynolds *et al.*, 2009). A recent analysis at CIMMYT and in the UK showed that genetic gains in yield potential may have fallen to 0.3% and 0.6% during the last 30 or 20 years, respectively (Fischer and Edmeades, 2010). To identify opportunities and tools to boost the genetic gains of yield potential, there is now an urgent requirement to understand more mechanistically the physiology of such a complex trait (Slafer, 2003; Fischer, 2007; Araus *et al.*, 2008).

Definition of the critical period for grain number generation and the assimilate supply for spike growth

Most yield variations are associated with those in grain number, both under different environments (Fischer, 1985; Slafer and Andrade, 1989; Savin and Slafer, 1991; Fischer, 1993; Magrin *et al.*, 1993) and as a result of genetic gains in yield potential (Slafer *et al.*, 1990; Fischer 2007). As grains would hardly compete for assimilates during post-anthesis (Miralles and Slafer, 1995; Borrás *et al.*, 2004; Acreche and Slafer, 2006), suggesting that sink strength during grain filling is the main factor limiting yield potential in wheat (Reynolds *et al.*, 2009, and references therein), further increments in grain number per unit area may increase yield potential (Miralles and Slafer, 2007).

The model generally used to understand the physiology of yield identifies critical periods within the crop cycle when the amount of available assimilates limits growth and yield (source limitation). For grain number in wheat, this period most frequently starts with the emergence of the penultimate leaf (20–30 d prior to anthesis) and ends at anthesis (Fischer, 1985; Fig. 1A). During this period, the rapid growth of spikes takes place, coinciding with a source-limited crop growth due to strong inter-plant competition (which is normally established at the onset of stem elongation) and with maximum rates of stem growth of each plant (Fischer and Stockman, 1980; Kirby, 1988; Siddique *et al.*, 1989; Fig. 1A). As the spikes bear the reproductive structures, it is not surprising that a strong positive relationship is usually observed between the number of grains and the spike dry weight at anthesis (Fig. 1B, for the experiments re-analysed in this paper), if differences in fruiting efficiency (FE; i.e. number of grains g^{-1} of spike: GN g spike^{-1} at anthesis) are not large. Based on that relationship, Fischer (1984) proposed understanding potential grain number (GN) as the product of spike dry weight at anthesis (SDW, g m^{-2}) and FE [equation 1]. SDW could be analysed as the product of duration of spike growth (DSG), crop growth rate (CGR g m^{-2} d^{-1}), and partitioning of dry matter to the spike or the spike partitioning index (SPI), all of them during pre-anthesis [equation 2]. The FE could be also determined as the product of the number of fertile florets per unit spike weight at anthesis and the number of grains per competent floret or grain set (GS). As modern cultivars have high grain set (>90% of fertile florets at anthesis set grains; Siddique *et al.*, 1989; González *et al.*, 2003a, 2005a), the number of fertile florets at anthesis (FF) essentially determines GN.

$$GN = SDW \times FE \qquad [1]$$

$$SDW = DSG \times CGR \times SPI \qquad [2]$$

This strong relationship between GN or FF and SDW has been shown to occur regardless of the treatments imposed to alter spike growth, for instance: shading (Fischer,1985; Thorne and Wood, 1987; Savin and Slafer, 1991; Abbate *et al.*, 1997; Demotes-Mainard *et al.*, 1999; Demotes-Mainard and Jeuffroy, 2004), nitrogen availability (Fischer, 1993; Abbate *et al.*, 1995; Demotes-Mainard *et al.*, 1999; Demotes-Mainard and Jeuffroy, 2001), changes in duration of the pre-anthesis period due to photoperiod treatments (Miralles *et al.*, 2000; González *et al.* 2003a, 2005a, c; Fischer, 2007; Serrago *et al.*, 2008), and genetic differences due to either the effect of dwarfing genes (Fischer and Stockman, 1980; Brooking and Kirby, 1981; Stockman *et al.*, 1983; Miralles *et al.*, 1998) or the year of release of the cultivars (Siddique *et al.*, 1989; Slafer and Andrade, 1993; Acreche *et al.*, 2008).

Although evidence of the relationship between number of fertile florets and spike dry weight at anthesis is abundant, the mechanistic bases of this relationship, i.e. the relationship between the development of floret primordia and the dynamics of spike growth, have rarely been studied. Thus, this paper was focused on establishing whether the survival of florets initiated is related to spike growth during pre-anthesis.

Dynamics of floret development and spike growth

Time to anthesis can be divided into three phases according to the organs being differentiated in the apical meristem: (i) the leaf primordia phase, from imbibition of the seed to floral initiation (FI), when the apical meristem differentiates

all leaves; (ii) the spikelet primordia phase, from FI to the formation of the terminal spikelet in the apical position (TS), when all spikelets are differentiated (Fig. 1C); and (iii) the floret primordia phase (FPP) or stem elongation phase (SE), from TS to anthesis (AN, anthers extruding from central spikelets), when most florets differentiate and develop within spikelets (Fig. 1A, D).

The spike has a great potential to differentiate floret primordia. Each spikelet initiates 6–11 florets, depending on its position on the spike, but most of these primordia do not complete their development and die before anthesis (Kirby, 1974, 1988; Fischer, 1984). The development of each floret primordium within a spikelet (from F1, the floret primordium most proximal to the rachis, to Fn, the floret primordium most distal from the rachis, both within the same spikelet position) can be studied using the scale developed by Waddington et al. (1983) (Fig. 1D). This scale identifies different stages of a floret primordium, from W3 (glume primordium present) to W10 (styles curved outwards with stigmatic branches spread wide and pollen grains present on stigmatic hairs) (Fig. 1D). The floret

primordia that do not fully develop to anthesis, first stop their development, and then anthers and ovaries lose volume and finally start dehydration (Fig. 1E), which is the criterion used to establish that a floret has died. Thus, in any spikelet there is a period of generation of potential floret primordia follow by a plateau of variable duration and then by a period of floret death.

As floret death has been reported to occur when the spike grows at maximum rate (Kirby, 1988; Fischer and Stockman, 1980; Siddique et al., 1989), it is usually considered that growth of the spike determines the number of florets that might progress normally. This hypothesis has been supported by breeding success in wheat yield, as it was mostly based on improvements in the number of grains m^{-2} (Calderini et al., 1999, and references therein), associated with an increased dry matter partitioning to the spikes during the floret primordia phase (Siddique et al., 1989; Slafer and Andrade, 1993). The specific analysis of the effects of semi-dwarfing genes (Rht) further supports the hypothesis, as a reduction in stem height resulted in a higher number of fertile florets as a consequence of an increased spike growth during pre-anthesis (Fischer and
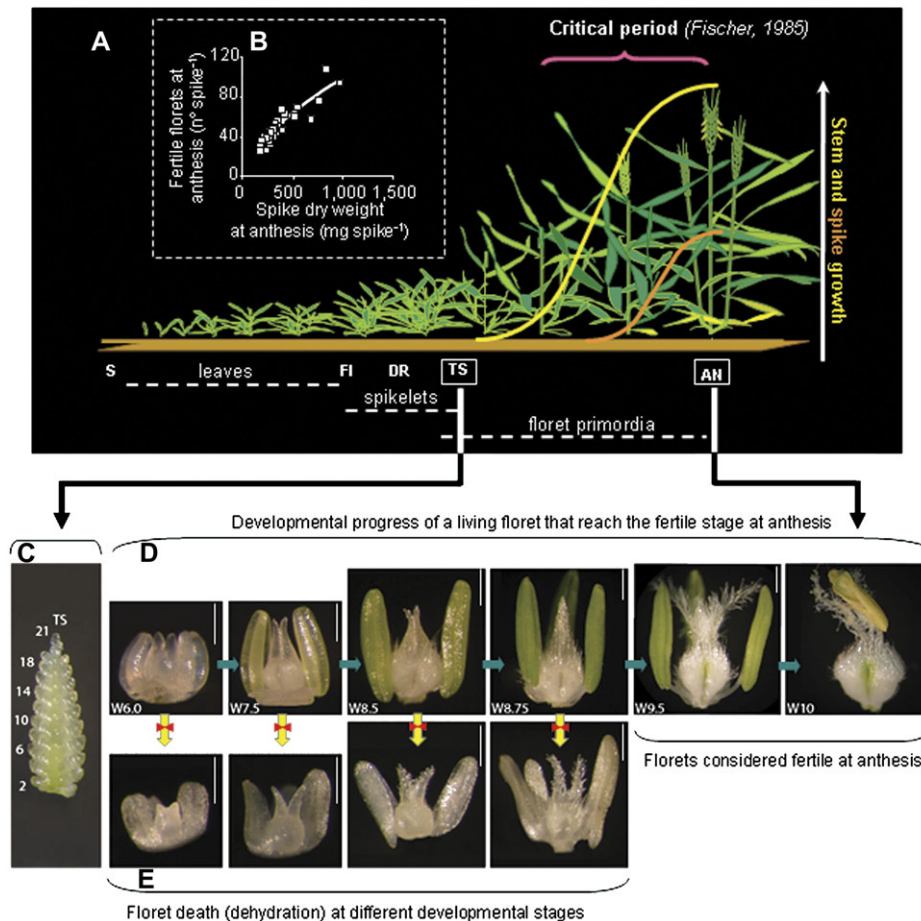
Fig. 1. Wheat cycle and critical period for grain number determination. (A) Wheat cycle showing the leaves, spikelets, and floret primordia phases and the critical period for grain number determination. S, sowing; FI, floral induction; DR, double ridge; TS, terminal spikelet; AN, anthesis. (B) Relationship between fertile florets and spike dry weight at anthesis for all cases analysed ($Y = -17.58(\pm4.72) + 3.68(\pm0.25)X^{0.5}$, $R^2 = 0.82$, $P < 0.001$, $n = 43$). (C) Spike at the terminal spikelet stage (2 mm long) with 21 spikelets. (D) Florets with normal development to anthesis (the Waddington stage (W-) is shown at the bottom left. The white line represents 0.1 mm). (E) Florets that will not survive to anthesis showing the first stages of dehydration.

Stockman, 1986; Siddique *et al.*, 1989; Miralles *et al.*, 1998). Exceptionally, some papers can be found reporting a small impact of the maximum number of floret primordia on the number of fertile florets at anthesis as well (Siddique *et al.*, 1989; Bancal, 2008). However, when isogenic lines for semi-dwarfing genes were studied, no difference in the maximum number of differentiated florets was observed, the survival of initiated floret primordia being the main cause of the increased number of fertile florets and grains during the green revolution in wheat (Siddique *et al.*, 1989; Miralles *et al.*, 1998). The importance of floret survival in setting the number of fertile florets at anthesis (without changes in maximum number of differentiated florets) was also highlighted when spike growth was altered by different growing conditions including shading during pre-anthesis (Fischer and Stockman, 1980), nitrogen (Sibony and Pinthus, 1988; Ferrante *et al.*, 2010), nitrogen and photoperiod treatments (Langer and Hanif, 1973), nitrogen and sowing dates (Whingwiri and Stern, 1982), and photoperiod and shading treatments during the floret primordia phase (González *et al.*, 2003b, 2005a).

The conceptual model relating the number of fertile florets and grains to pre-anthesis spike growth was recently questioned (Bancal, 2008, 2009; Ugarte *et al.*, 2010). Based mainly on (i) a positive relation between grain number and stem +sheaths dry matter at harvest (Bancal 2008), (ii) the reduction in the number of fertile florets at anthesis due to low R/FR ratios not associated with increased stem growth (Ugarte *et al.*, 2010), (iii) the apparent delay of the onset of floret death in relation to the beginning of spike growth (Bancal, 2008), and (iv) the onset of floret death associated with certain stages of floret development in the central spikelets (Bancal, 2009), it was concluded that (a) there is a positive contribution of stem growth to grain number (Bancal, 2008) and spike:stem competition may not determine grain number (Bancal, 2008) or fertile florets at anthesis (Ugarte *et al.*, 2010), and (b) the onset of floret death may instead be a developmental process that is not associated with

spike growth (Bancal, 2009). It is important to understand the processes determining fertile florets (and grain number) not only for predicting the impact of management practices but also for identifying beneficial traits for future breeding to raise yield potential. The aim was to validate the model of assimilate limitation during the critical period from a detailed re-analysis of floret development and dynamics of spike growth in a set of experiments that included different cultivar types, experimental approaches, and type of treatments imposed during pre-anthesis to alter spike growth.

Materials and methods

Set of experiments for re-analysis

The data used in this paper for re-analyses were collected in six independent experiments, one under controlled conditions and five in the field, carried out in six different growing seasons (1997, 2000, 2001, 2002, 2005, and 2006; Table 1). The cultivars included spring and winter, semi-dwarf and tall, and photoperiod-sensitive and -insensitive types, from breeding programmes of different countries (Australia, Argentina, and the UK). Wheat responds to photoperiod as a long day plant, i.e. accelerating rate of development as the photoperiod increases. During the floret primordia phase (or stem elongation phase, from TS to AN) a range of photoperiod treatments were applied to alter spike growth (mainly by changing its growing period, DSG in equation 2). For the growth chamber experiment, the photoperiod treatments consisted of reciprocal changes between different daylengths. Plants were grown at 9, 13 or 19 h from sowing to terminal spikelet and from then to anthesis one-third of the plants remained in the same photoperiod and the other two-thirds were reciprocally interchanged to the other two photoperiods (details in Miralles *et al.*, 2000). For the field experiments, the treatments were applied only during the floret primordia phase and consisted of (i) the natural photoperiod of the growing season (NP+0) or extensions of 3 h (NP+3) or 6 h (NP+6) over the NP, and (ii) the natural photoperiod shortened by 3 h (NP–3). For photoperiod extensions, portable lighting systems of low intensity were installed over the field plots (details in González *et al.*, 2003a), while for photoperiod reduction a light shelter was mounted on fixed rails over the field plots delaying sunrise by 75 min and

Table 1. Description of the experiments used for the re-analyses

SD, semi-dwarf; SH, standard height; ST, spring type; SRV, slightly responsive to vernalization; RV, responsive to vernalization; WT, winter type; PS, photoperiod-sensitive; PI, photoperiod-insensitive (*Ppd-D1*).

Origin	Year	Cultivar	Description	Experiment and treatments
Miralles *et al.* 2000	1997	UQ189	SD, ST, PS	Growth chamber (phytotron), photoperiod transferences at terminal spikelet (9, 13 or 19 h)
González *et al.* 2003b	2000	Eureka Ferrocarril Sur	SH, SRV, PS	Field, NP+0/NP+6 from terminal spikelet to anthesis
		Buck Manantial	SH, ST, PS	
González *et al.* unpublished	2000	ProINTA Puntal	SD, RV, PS	Field, NP+0/NP+6 from terminal spikelet to anthesis
González *et al.* unpublished	2001	Mercia (*recessive*)	SH, WT, PS	Field, NP+0/NP+6 from terminal spikelet to anthesis
		Mercia (*Ppd-D1*)	SH, WT, PI	
		Cappelle Desprez (*recessive*)	SH, WT, PS	
		Cappelle Desprez (*Ppd-D1*)	SH, WT, PI	
González *et al.* 2005b	2002	Buck Manantial	SH, ST, PS	Field, shading/control and NP+0/NP+6 from terminal spikelet to anthesis
Vázquez *et al.* unpublished	2005	Mercia (*recessive*)	SH, WT, PS	Field, shading/control, and NP–3/NP+0/NP+3 from terminal spikelet to anthesis
		Mercia (*Ppd-D1*)	SH, WT, PI	
Vázquez *et al.* unpublished	2006	Mercia (*recessive*)	SH, WT, PS	Field, shading/control, and NP–3/NP+0/NP+3 from terminal spikelet to anthesis
		Mercia (*Ppd-D1*)	SH, WT, PI	

advancing sunset by 75 min (the other 0.5 h were civil twilight periods in which the shelter was closed as well). Both the light shelter and the portable lighting structures had negligible effects on daily incident radiation. To alter spike growth without altering development, in some experiments photoperiod treatments were combined with shading during the floret primordia phase, using black nets intercepting 67% of the incoming photosynthetic active radiation over the canopy without altering canopy temperature (details in González et al., 2005b).

In all cases, the dynamics of spike growth and floret development were studied in detail through frequent sampling (twice or thrice weekly). The process of floret primordia dehydration was taken into account to consider whether a floret had started the degeneration process leading to floret death or was still developing normally towards the stage of fertile floret (Fig. 1). Three spikelet positions along the spike were analysed: apical (second/third spikelet from the tip of the spike), central (spikelet in the middle), and basal (third/fourth spikelet form the base) (Fig. 1C).

The Table Curve 2D Program (Anonymous, 1991) was used for fitting regressions and the P values were calculated as Probability (Pr) $Y \geqslant X$.

Re-analysis of floret primordia generation and death

For each spikelet position, bi-linear threshold functions were used to describe the progress of living florets (those that did not show dehydration) once the maximum number of florets was reached and during the degeneration phase (Fig. 3A). To calculate the maximum number of living floret primordia (MaxNFP), rate of floret death (RFD), and floret survival percentage (SF) equation [3] was used (Fig. 3A), where Y=the number of living florets per spikelet and X=the duration of the floret primordia phase.

$$Y = A - BX(X \geqslant C) - (BC(X<C)) \qquad [3]$$

The MaxNFP is the maximum value achieved by equation [3] (Funct MAX), RFD is the parameter B, and SF was calculated as the ratio between the number of fertile florets (which is the minimum number of living floret primordia at the end of floret death, MinNFP) and MaxNFP [SF=(Funct MIN/Funct MAX)×100]. Parameter C of equation [3] indicates the onset of floret death. In the few cases where the dynamics of the number of living florets did not show a plateau (i.e. floret initiation was immediately followed by floret death), equation [4] was used instead.

$$Y = A + BX(X \leqslant C) + BC(X \geqslant C) + D(X-C) \qquad [4]$$

In that case, RFD was estimated as |D| and the onset of floret death as the parameter C.

For analysing the data of all the different treatments and studies together, the values of living florets were standardized as a proportion of the MaxNFP within each spikelet, and then equation [3] or [4] was fitted again (Fig. 3B). Rate of floret death in this analysis is expressed as the relative number of floret primordia per unit of thermal time (RNFP [°Cd]$^{-1}$, Tb=0 °C). The duration of the floret primordia phase (x-axis) is expressed either in thermal time or in relative terms (from 0–100% in each case). Alternatively, the time (x-axis) was measured as the progress in the developmental stage of the most proximal floret (F1) of the central spikelet (which is the floret in the spike with most advanced development, Fig. 2C), using the scale of Waddington et al. (1983).
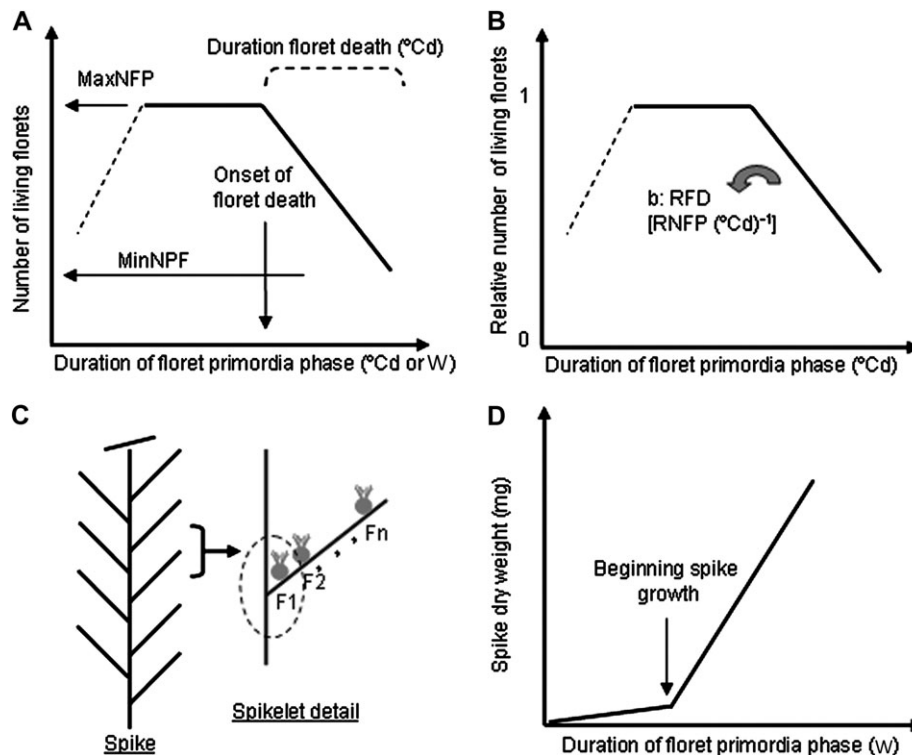

Fig. 2. Schematic representation. (A) Relation between the number of floret primordia and duration of the floret primordia phase showing the estimation of (i) the maximum number of floret primordia differentiated (MaxNFP), (ii) the number of fertile florets at anthesis or living florets (MinNFP), (iii) the onset of floret death, and (iv) the duration of the floret death period. (B) Relation between the relative number of floret primordia and duration of floret primordia phase showing the estimation of the rate of floret death (RFD). (C) Detail of the spike and the central spikelet showing the proximal (F1, F2) and distal (Fn) positions of the floret primordia along the spikelet. (D) Relation between spike dry weight and duration of the floret primordia phase showing the estimation of the beginning of spike growth at maximum rate.

Re-analysis of spike growth

To analyse spike growth, a bi-linear threshold equation was fitted (Fig. 2D).

$$Y = A + BX(X <= C) + BC(X > C) + D(X - C)(X > C) \quad [5]$$

where Y=spike dry weight (mg), X= relative duration of the floret primordia phase (from 0–100% within each case or the Waddington stage of F1 in the central spikelet), B=slow spike growth rate, D=rapid spike growth rate, C=breakpoint between B and D.

Results and discussion

Floret primordia generation and death, number of differentiated primordia, and of fertile florets

The fitting of equations [3] and [4] were statically significant in all of the 82 floret primordia dynamics analysed (R^2 ranged from 0.56 to 0.99, P <0.05; and for 80% of these cases R^2 >0.71; for details see Supplementary Table S1 at *JXB* online). Floret primordia were increasingly differentiated until reaching a maximum, which, in most cases, was held for a limited time (plateau) and then many of the developing florets died while few survived to produce fertile florets (Fig. 3A; Kirby, 1988). The onset of floret death ranged from 25–75% of the relative duration of the floret primordia phase, and was co-ordinated between the different spikelets along the spike (Fig. 3B). Thus, even though floret primordia initiation starts earlier in central than in apical or basal spikelets (Kirby, 1974), floret death starts almost simultaneously in all spikelets. The great variation observed in the onset of floret death (in relative terms as well as in thermal time) was associated with the large differences in the duration of the floret primordia phase explored with our data set, from 479 °Cd to 1118 °Cd, due to the combination of different cultivars and photoperiod sensitivity genes and treatments. If only natural field conditions are considered (i.e. only NP+0 treatments) the range of variation decreased noticeably but it remained important, from 479 °Cd to 762 °Cd. As the duration of FPP increased, the onset of floret death was delayed (Fig. 4A) and the period of floret death increased (Fig. 4B). These relationships were evident under field conditions as well as in the growth chamber experiment. In this last case, UQ189 showed, in general, an earlier onset of floret death and a longer duration of the period of floret death (Fig. 4A, B). The variation in the onset of floret death may be partially explained by photoperiod responses during the floret primordia phase. In addition to the duration of the FPP responding to average photoperiod in the sensitive cultivars (Fig. 4C), so also did the timing of floret death onset (Fig. 4D). This response can clearly be seen in the two most photoperiod-sensitive cultivars: Buck Manantial, which is a spring type highly sensitive to photoperiod and Mercia$_{recessive}$ vernalized for 50 d (Fig. 4C, D).

In the six studies reported, and for each of the apical, central, and basal spikelet positions, there was no relationship between the number of fertile florets at anthesis and the maximum number of differentiated florets (Fig. 5A), for floret survival ranging from zero (none of the primordia developed to produce a fertile floret) to 64%. By contrast, a positive relationship between the number of fertile florets at anthesis and the survival of floret primordia was observed, independently of the spikelet position, explaining 85% of the variation (Fig. 5B). These results strengthen the overwhelming role of floret primordia survival, over floret differentiation, for setting the number of fertile florets at anthesis (Langer and Hanif, 1973; Fischer and Stockman, 1980; Whingwiri and Stern, 1982; Sibony and Pinthus, 1988; González *et al.*, 2003*b*, 2005*a*; Ferrante *et al.*, 2010). In this context, understanding what determines floret primordia survival may be instrumental in the further improvement of wheat yield.

Floret survival could be understood as a consequence of rate of floret death and duration of floret death. As far as we are aware, floret survival was never analysed in this way. Floret survival was mostly determined by the rate of floret death, explaining 30% of the variation (Fig. 5C). Although a unique regression was fitted for the three spikelet positions, the maximum rates of floret death (and the minimum values of floret survival) were observed in the
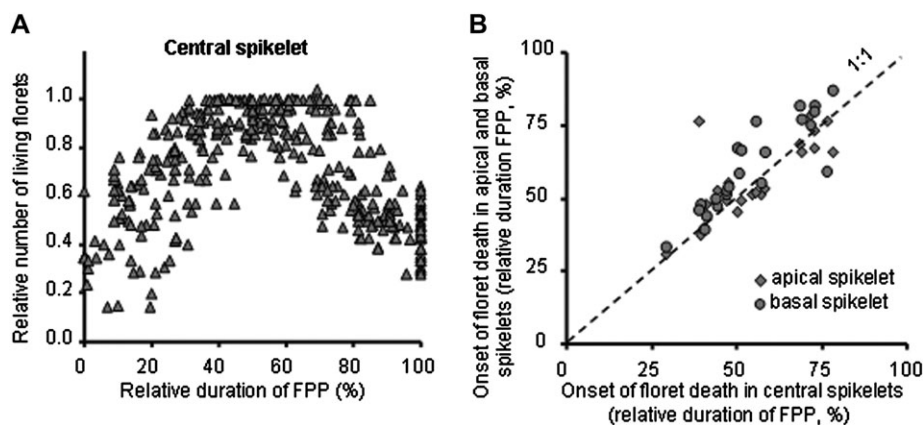

Fig. 3. Development and death of floret primordia. (A) Relative number of living florets in central spikelets versus relative duration of floret primordia phase (*n*=36). (B) Relationship between onset of floret death in apical/basal versus central spikelets (*n*=48). All experiments shown.
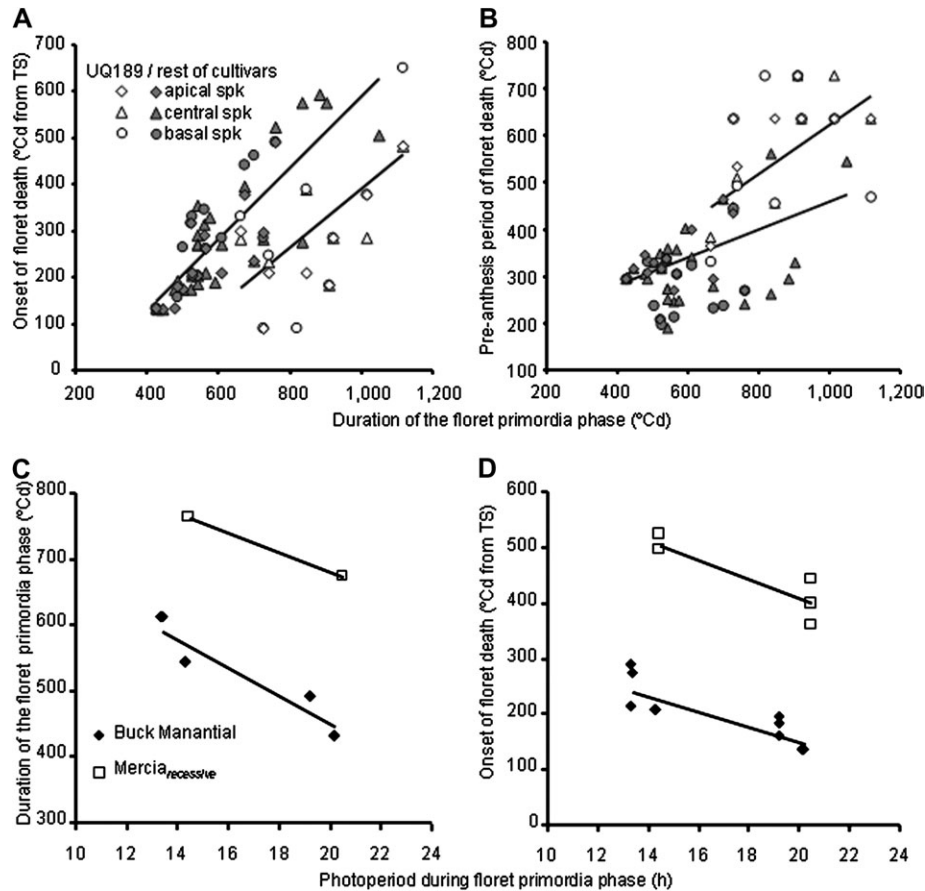
Fig. 4. Onset and period of floret death. (A) Relation between onset of floret death and duration of the floret primordia phase ($Y=102.4(\pm47.1)+2.6E\text{-}07(\pm5.9E\text{-}08)X^3$, $R^2=0.41$, $P<0.001$, $n=23$, for UQ189; $Y=-176.9(\pm42.6)+0.76(\pm0.07)X$, $R^2=0.66$, $P<0.001$, $n=58$ for the rest of the cultivars). (B) Relation between duration of the pre-anthesis period of floret death and duration of the floret primordia phase ($Y=813.1(\pm678)-161140000(\pm43194300)/X^2$, $R^2=0.32$, $P<0.005$, $n=23$ for UQ189, $Y=274(\pm14)+1.7E\text{-}07(\pm4.7E\text{-}08)$ X^3, $R^2=0.16$, $P<0.001$, $n=58$). (C) Relation between duration of floret primordia phase and photoperiod explored during that phase ($Y=873(\pm41)-21.2(\pm2.4)$, $R^2=0.86$, $P<0.001$, $n=11$ for Buck Manantial; $n=5$ for Mercia$_{recessive}$ fitted by hand). (D) Relation between onset of floret death and photoperiod explored during floret primordia phase ($Y=426(\pm46)-13.9(\pm2.7)$, $R^2=0.66$, $P<0.001$ $n=11$ for Buck Manantial, and $Y=755(\pm76)-17.5(\pm4.3)$, $R^2=0.67$, $P<0.05$, $n=5$ for Mercia$_{recessive}$).

basal spikelets. These results provide support to the suggestion that basal spikelets would have less priority in assimilate partitioning than the central and apical spikelets (Stockman *et al.*, 1983), explaining why they are the first to reduce the number of fertile florets when there is a shortage of assimilates prior to anthesis (Stockman *et al.*, 1983; Sybony and Pinthus, 1988; Craufurd and Cartwright, 1989). Duration of the period of floret death had a positive effect on floret primordia survival mediated by its negative relationship with the rate of floret death (Fig. 5D). This relationship was detected across the wide range in duration of the floret primordia phase explored with the data set (from 479 °Cd to 1118 °Cd). As duration of the floret primordia phase and duration of the period of floret death increased (Fig. 4B), the rate of floret death decreased yielding higher floret survival. These results may explain and expand our previous suggestion that the number of fertile florets may depend on the time available for the development and growth of the florets (González *et al.*, 2005b). The likelihood of a floret primordium becoming

a fertile floret is determined mostly during the period of floret death and the longer the period, the higher the likelihood of survival. Based on these results it could be stated that the impact of photoperiod on floret survival is not minor. However, spike growth was analysed during FPP with the same detail as before in order to draw a clearer picture of the likely dependence or independence of floret death on spike growth.

Supporting the assimilate supply model for floret survival

Previous suggestions by our group and other researchers, that floret death might be inversely related to spike growth, were based on the facts that both processes occur more or less simultaneously and that, at anthesis, there is a good relationship between the number of fertile florets (or grains at maturity) and spike dry weight (Fischer and Stockman, 1980; Kirby, 1988; Siddique *et al.*, 1989; González *et al.*, 2003b, 2005b). However, very few attempts were actually
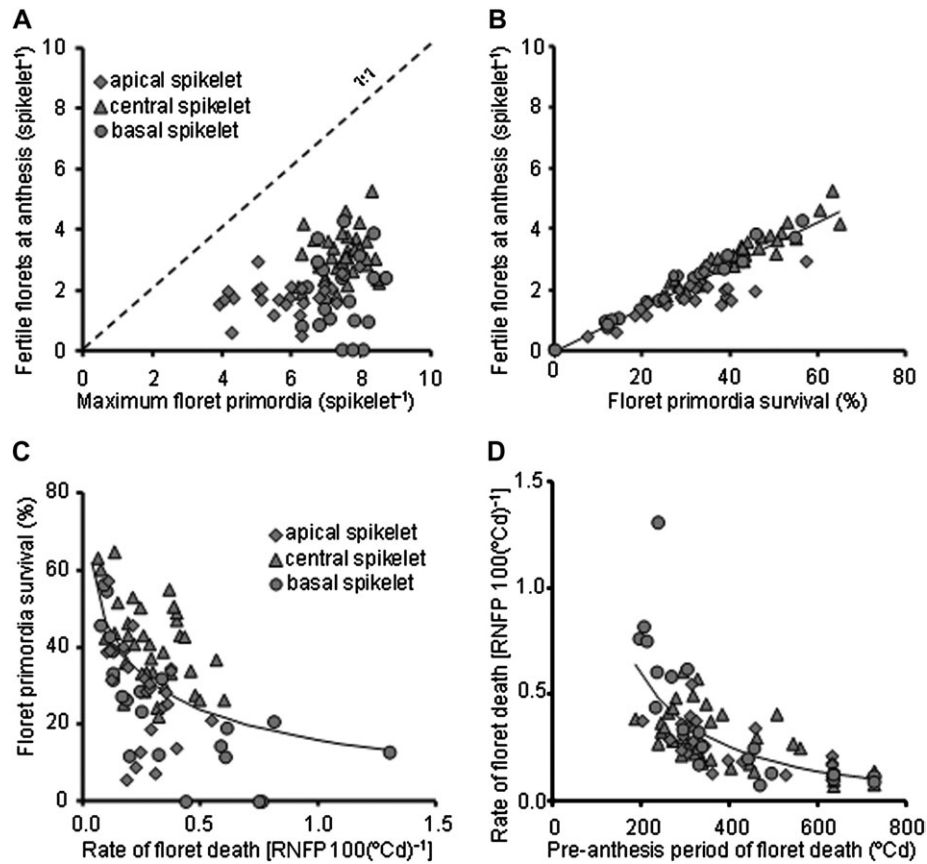
Fig. 5. Fertile florets at anthesis, maximum number of differentiated florets, and survival. Relationship between the number of fertile florets at anthesis and (A) the maximum number of floret primordia ($n=79$); and (B) the floret primordia survival ($Y= -0.034(\pm0.119)+0.071(\pm0.003)X$, $R^2=0.85$, $P <0.001$, $n=82$). (C) Relationship between floret primordia survival and rate of floret death ($Y= -8.57(\pm6.83)+1.15(\pm0.18)(\ln X)^2$, $R^2=0.30$, $P <0.001$, $n=82$). (D) Relationship between rate of floret death and duration of the pre-anthesis period of floret death ($Y= -8.8\text{E-}04(\pm5.1\text{E-}04)+1.37(\pm0.16)/X$, $R^2=0.44$, $P <0.001$, $n=82$).

made to quantify the relationship between the fate of floret primordia and spike growth immediately before anthesis. There are recent reports suggesting that spike:stem competition may not determine grain number or fertile florets (Bancal, 2008; Ugarte *et al.*, 2010) and that the onset of floret death may instead be a developmental process that is not associated with spike growth (Bancal, 2009). If this were so, the relationship between number of fertile florets or grains and spike dry weight at anthesis would be fairly casual, and therefore searching for ways to increase spike growth in order to improve wheat yield potential further (Slafer *et al.*, 2005; Reynolds *et al.*, 2009; Foulkes *et al.*, 2010) would largely be irrelevant.

Ugarte *et al.* (2010) studied the response of spike growth and floret development to changes in the R/FR ratio (red/far red) during the floret primordia phase. The hypothesis was that a low R/FR ratio would increase plant stature (and stem growth), decreasing spike weight and fertile florets at anthesis. As a low R/FR ratio effectively decreased spike growth and fertile florets, but also reduced peduncle growth, the conclusion was that floret death was not driven by spike growth. By contrast, it is considered that, despite the fact that competition between the growing spike and stem was unexpectedly not increased by the low R/FR ratio,

the floret number was still related to growth of the spike, as a decreased spike growth was associated with decreased fertile florets at anthesis. Although the dynamics of spike growth and the number of living florets were measured (Ugarte *et al.*, 2010), it is hard to analyse from the data collected if the onset of floret death was associated with the beginning of spike growth because measurements started once the spike was growing at its most rapid rate.

On the other hand, Bancal (2008, 2009) studied in detail both the fate of floret primordia and the dynamics of dry matter allocation to the spikes and stems during pre-anthesis in six French cultivars. The first conclusion that spike:stem competition may not determine grain number was based mainly on the positive relationship observed between grain number per spike and stem+sheaths dry mater at harvest (see Fig. 1D in Bancal, 2008). According to this study, if competence between spike and stem growth determines the number of living florets and grains, then a negative relationship should have been found (Bancal, 2008). In the same paper, however, a good positive association between grain number per spike and spike chaff dry matter at harvest (which is considered a 'proxy' to spike dry weight at anthesis) was observed (Fig. 1A in Bancal, 2008). The treatments applied to modify spike dry weight

were different population density and, during the floret primordia phase, shading and photoperiod. As dry matter partitioning is not greatly modified by any of these treatments, as it is essentially a genotypic attribute (Fischer, 1984), both spike and stem growth changed in parallel, explaining the positive correlation between the number of reproductive organs and dry matter of both spike and stem. This would be reinforced by the fact that the only cultivar that did not fit to the relationship between grain number per spike and stem+sheaths dry matter was the old tall cultivar (with the smallest spike:stem ratio), which showed for the same range of stem+sheath dry matter fewer grain numbers per spike (Fig. 1D in Bancal, 2008) due to less spike chaff dry matter (Fig. 1A in Bancal, 2008).

The second conclusion that the onset of floret death may instead be a developmental process that is not associated with spike growth (Bancal, 2008, 2009) was initially based on the fact that the onset of floret death started c. 55 ± 14 °Cd earlier than spike growth at the maximum rate. Bancal (2008) analysed spike growth through an asymmetric sigmoid curve and assessed the growth rate as the first order derivate, then relating the inflection point of the spike growth curve, as the point of maximum rate, to the onset of floret primordia death. Although this approach is methodologically appropriate for general growth analysis, it might have been inappropriate for identifying whether the beginning of rapid spike growth precedes or follows the onset of floret death. It seems unsound to propose that the growth of the spikes until they reach half of their final weight at anthesis is totally unnoticed by the floret primordia developing in these spikes. Thus, the supposed mismatching reported between the onset of floret death and the beginning of rapid spike growth may only be reflecting the assumption in the analysis concerning the precise stage at which accelerating spike growth may affect floret development. Assuming that floret development is sensitive to spike growth before it has c. 50% of its final weight could equally have driven one to the opposite conclusion, i.e. that onset of floret death is only triggered after the beginning of rapid spike growth. Although a sigmoidal function can naturally fit well data of spike growth before anthesis, the visual inspection of spike growth reveals a bilinear pattern: the spike grows first at a relatively slow rate and then at a considerably higher rate until anthesis (Fig. 6A). Due to the large variations in treatments, this data set includes a very wide range of spike dry weight at anthesis ranging almost 6-fold, from 0.17 to 1.2 g spike^{-1} (see Supplementary Table S2 at *JXB* online). Instead of assuming the onset of rapid spike growth as the first order derivate of the sigmoidal curve (Bancal, 2008), when already half of the maximum spike weight at anthesis had been achieved, the timing of beginning of rapid spike growth has been redefined in this paper as the breakpoint between the two lines of equation [5] (parameter C, Fig. 2C). This represents the actual time when spikes start growing at a substantially higher rate than before, and most frequently occurs when the spikes had accumulated less than c. 20% of their final weight at anthesis. The fitting of the model yielded $R^2 >$
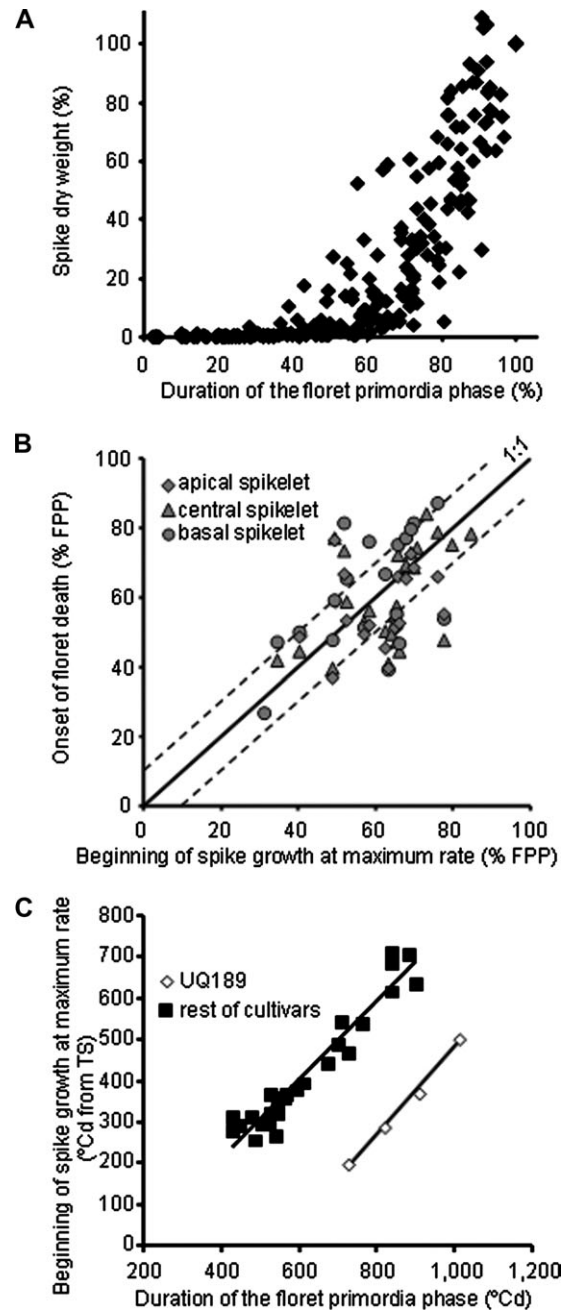

Fig. 6. Spike growth at maximum rate and onset of floret death. (A) Spike growth of the analysed data showing the bilinear pattern (n=28). (B) Onset of floret death versus beginning of spike growth at maximum rate (equation [5], n=63, time as percentage duration of the floret primordia phase). (C) Relation between the beginning of spike growth at the maximum rate and duration of the floret primordia phase for UQ189 ($Y= -570\ (\pm49)+1.05(\pm0.06)X$, R^2=0.98, P <0.001, n=4) and the rest of the cultivars ($Y= -170(\pm35)+0.95(\pm0.05)X$, R^2=0.91, P <0.001, n=25).

0.86, P <0.05 for all of the cases analysed (n=28; see Supplementary Table S2 at *JXB* online). When the onset of floret death was related to the beginning of spike growth at maximum rate as defined by the breakpoint, 73% of the cases analysed aligned within a 10% margin of the 1:1 ratio (Fig. 6B). The same analysis applied to Bancal's data

(cultivar Tremie; Bancal, 2008) also shows that the onset of floret death followed the beginning of spike growth at maximum rate. The beginning of spike growth at maximum rate was delayed as the duration of floret primordia phase increased (Fig. 6C), similar to the onset of floret death (Fig. 4A), highlighting the close association between these two processes (note the cultivar's variation given by UQ189). In a previous paper (Gonzalez *et al.*, 2005b) it was suggested that the beginning of spike growth may change in response to the photoperiod explored during the floret primordia phase, explaining at least part of the results observed in the present analyses.

As some cases (*c.* 20%) may be below the 1:1 ratio (even with the breakpoint analysis), it was speculated that some direct effects of photoperiod might have been involved in the onset of floret death (González *et al.*, 2003b), suggesting that floret death might be induced by developmental processes rather than by assimilate supply to spike growth (González *et al.*, 2003b). To identify more clearly those supposed effects, shading and photoperiod treatments were combined during the floret primordia phase, concluding that degeneration of more distal florets within spikelets (those that never do contribute to the number of fertile florets at anthesis; i.e. not even in the highest-yielding conditions do these primordia become fertile) seemed to occur before beginning of spike growth at maximum rate (Gonzalez *et al.*, 2005a,b). However, the onset of floret death of those florets in the middle of the spikelet (which are the ones that contribute differentially to the number of fertile florets at anthesis, depending on the environment), just coincided with the beginning of spike growth at maximum rate (Gonzalez *et al.*, 2005b); explaining the strong relationship observed between the number of fertile florets and spike dry weight at anthesis, independently of the treatment applied to alter spike growth, photoperiod (development) or shading (growth) (Gonzalez *et al.*, 2005a).

Previous studies observed that floret death started when the floret most proximal to the rachis (F1) of the central spikelets reached stage W8 (stigmatic branches elongating) (Craufurd and Cartwright, 1989) or a stage ranging between W7 (styles elongating) to W8 (Bancal, 2009). These results allowed the conclusion that a particular floret stage of the most advanced floret primordia of the spikes may determine the onset of death of less developed florets. This hypothesis implies that the onset of floret death might not be related to a shortage of assimilates for spike growth but, instead, may exclusively be a developmental process (Bancal, 2009); although it would not preclude the relevance of spike growth on the rate of floret death once the process started. To test this hypothesis, equations [3], [4], and [5] were fitted again to our data set but using the stage of the most advanced floret (F1 of central spikelet) as the time scale. The fitting of these new curves for floret primordia development (equations [3] and [4]) resulted in $R^2 > 0.70$, $P < 0.05$ for more than 75% of the cases analysed ($n=70$, R^2 range from 0.47 to 0.99, $P < 0.05$; see Supplementary Table S3 at *JXB* online). For spike growth (equation [5]), the fitting of the curves showed $R^2 > 0.90$, $P < 0.05$ for *c.*

80% of the cases analysed ($n=26$, R^2 range from 0.61 to 0.99, $P < 0.05$; see Supplementary Table S4 at *JXB* online). The dynamics of living florets was very similar for all cases and spikelets (Fig. 7A) and the F1 stage when floret death began ranged between W8 and W9 (stigmatic branches erect and hairs on ovary wall) for 70% of the cases in central spikelets and 80% of the cases in apical and basal spikelets. Re-analysing the spike growth data showed that the beginning of spike growth at the maximum rate took place when the F1 was between W8 and W9 in 100% of the cases (Fig. 7B), conflicting with the idea that the onset of floret death might not be associated with spike growth (Bancal, 2009). If instead of considering the spike dry matter, the amount of water-soluble carbohydrates in the spike were considered, the idea that the onset of floret death is a consequence of assimilate starvation is reinforced. These carbohydrates reach maximum values in the early stages of spike growth (when it is *c.* 10% of its final weight) followed by a steady decline (Fischer and Stockman, 1980; Stockman *et al.*, 1983). Only three studies focusing on the dynamics of living florets also measured the water-soluble carbohydrates (Fischer and Stockman, 1980; Bancal, 2008; Ghiglione *et al.*, 2008). In the three cases, the concentration of these carbohydrates was highest before the beginning of spike growth at maximum rate (and before the onset of floret death as well), and decreased when the spike started to grow, followed by the onset of floret death, implying that carbohydrate starvation may be involved in triggering floret
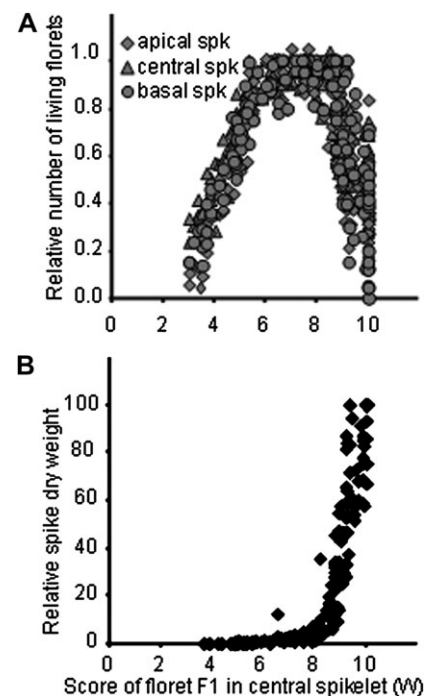

Fig. 7. Dynamics of spike growth and floret death measured through floret development. (A) Relative number of living florets ($n=52$) versus the developmental score (Waddington) of the most advance floret (F1) in central spikelet. (B) Relative spike dry weight ($n=26$) versus the developmental score (Waddington) of the most advance floret (F1) in central spikelet.
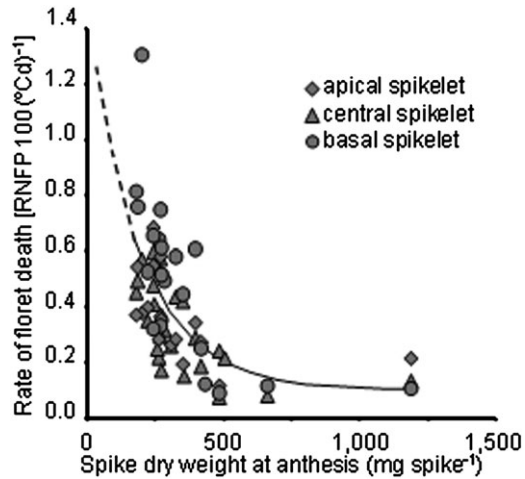
Fig. 8. Rate of floret death and spike growth. Relationship between the rate of floret death and the spike dry weight at anthesis ($Y=9.9E\text{-}04(\pm 8.8E\text{-}04)+0.013(\pm 0.004)EXP(-X/C)$, $R^2=0.39$, $P<0.001$, $n=63$).

death. Not only was the onset of floret death associated with the beginning of spike growth, but the relationship also seemed to have a clear quantitative component: as final spike growth at anthesis increased, rate of floret death decreased in all spikelet positions (Fig. 8). The asymptotic nature of this relationship suggests that the increasing survival of floret primordia would require increasing amounts of assimilate availability. This extra requirement may be related to the fact that distal florets within spikelets are not connected to the main vascular bundles of the rachilla but to a sub-vascular system (Hanif and Langer, 1972).

Conclusion

In this article, the model of assimilate supply for spike growth as a main determinant of the number of fertile florets at anthesis was validated from the re-analysis of the functional process driving the strong relationship between FF and SDW at anthesis, i.e. the dynamics of spike growth and floret development and survival during pre-anthesis. Although the alternative hypothesis that the onset of floret death may instead be a developmental process could be proposed (given the complex nature of the process of floret development and the fact that, in 20% of the cases analysed, the onset of floret death was earlier than the beginning of spike growth at maximum rate), in *c.* 80% of the cases analysed, the onset of floret death was associated with the beginning of spike growth at maximum rate, independently of the time-scale measurement (relative duration or development of the F1 in the central spikelet) used for analyses. Not only was the onset of floret death associated with spike growth but also the rate of floret death (the main determinant of floret survival) showed a negative quantitative relationship with the spike weight obtained at anthesis. The idea that fertile florets at anthesis are not determined by assimilates diverted to the spike (Bancal, 2008; Ugarte *et al.*, 2010) was not supported (and in fact re-interpreting

data from these two papers can support the assimilate supply model as well). Therefore, focusing on traits associated with a promoted spike growth is valuable when breeding for further raising yield potential in wheat.

Supplementary data

Supplementary data can be found at *JXB* online.

Supplementary Table S1. Floret primordia death in central (ST 1 A), basal (ST 1 B), and apical (ST 1 C) spikelets of the spike. Number of living floret primordia versus duration (°Cd) of the floret primordia phase.

Supplementary Table S2. Spike dry weight (mg) versus relative duration of floret primordia phase (%).

Supplementary Table S3. Floret primordia death in central (ST 3 A), basal (ST 3 B), and apical (ST 3 C) spikelets of the spike. Number of living floret primordia versus Waddington score of the most advanced floret, e.g. F1 central spikelet.

Supplementary Table S4. Relationship between spike weight (mg) and Waddington score of the most advanced floret, e.g. F1 central spikelet.

Acknowledgements

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