



Floret development and spike fertility in wheat: Differences between cultivars of contrasting yield potential and their sensitivity to photoperiod and soil N

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ABSTRACT

In a previous paper (Field Crops Res. 203, 114–127), we showed that the difference in yield potential between a contemporary and a traditional cultivar was due to differences in fruiting efficiency, likely derived from differences in spike fertility (fertile florets per spike) while having similar spike dry weights at anthesis. In this study, we determined the mechanistic bases of these genotypic differences in spike fertility analysing the initiation of all floret primordia per spike (up to 8), the maximum number of florets initiated per spikelet, and the associated floret developmental rates and their fate to become fertile florets under contrasting photoperiod (natural vs extended) and nitrogen availability (50 or 200 kg N ha⁻¹) during the stem elongation phase. Under potential growing conditions (natural photoperiod, high nitrogen availability), the contemporary cultivar owed its higher spike fertility to the improved rate of floret development, which mainly determined an improved level of floret primordia survival to produce fertile florets. The sensitivity of the floret developmental patterns to faster development due to exposure to an extended photoperiod and, to a larger extent, a reduction in N availability was similar for both cultivars, providing a basis for the consistent differences in spike fertility across a range of environments. The response again determined a main effect through increasing floret mortality reducing therefore the level of fertile florets per spikelet in these conditions.

1. Introduction

As genetic gains in yield strongly depend upon further raising grain number per m² (Sadras and Slafer, 2012; Slafer et al., 2014), understanding the physiological bases of the determinants of grain number is instrumental to design better management strategies or breeding programs (Fischer, 2011, 2007; Foulkes et al., 2011). Spike fertility (i.e. the final outcome of the processes of floret initiation and survival determining the number of fertile florets per spike) is a critical attribute determining grain number in wheat (Foulkes et al., 2011; González-Navarro et al., 2016). Spike fertility in turn varies between genotypes (e.g. Slafer and Andrade, 1993) and responds to resources (e.g. nitrogen availability: Ferrante et al., 2013a, 2010; elevated CO₂: Dias de Oliveira et al., 2015) and signals (e.g. daylength: González et al., 2005, 2003). Naturally, the response of spike fertility must have been the consequence of the effects of N, CO₂ and daylength on floret developmental processes. Indeed, the effects of treatments on the final number of

fertile florets of a wheat spike is an outcome of an organogenesis process characterised by a massive initiation of floret primordia followed by a phase of strong mortality of many of the initiated primordia (Kirby, 1988), regardless of whether the effects are genotypic (González-Navarro et al., 2015; Guo et al., 2016; Miralles et al., 1998; Prieto et al., 2018a) or environmental due to availability of resources (González et al., 2011; Serrago et al., 2008) or signals (Pérez-Gianmarco et al., 2018; Prieto et al., 2018b). Although the initiation of floret primordia seems a purely developmental process, the survival of primordia to produce fertile florets (most of which become grains and determine yield; Fischer, 2008; Slafer, 2003) seems to be strongly related to the dry matter accumulation in the spikes, c. 20–30 days immediately before anthesis, in which floret development occurs (Fischer, 1985). In fact, the triggering of the floret primordia mortality phase seems to follow a trophic model, relating the onset of floret death with the initiation of active growth of the juvenile spikes (Ferrante et al., 2013b; González et al., 2011; Slafer et al., 2005).

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In a previous study, Fischer and Stockman (1986) showed differences in grains number per spike between tall and dwarf wheat isolines with contrasted photoperiod regimes under controlled-environment. Since that, as far as we are aware, there were no more studies in the literature comparing the responses of floret development of wheat cultivars differing in yield potential, beyond the effect of semi-dwarfism, and even less on their responses to nitrogen (N) availabilities or photoperiod. Therefore, it seems not simple to establish whether differences in spike fertility between wheat genotypes of different yield potential are constitutive (i.e. a trait that is characteristic of a genotype and its relative performance is not altered by the growing condition) or otherwise (they would depend strongly on the growing condition). Naturally spike fertility would always respond to changes in the environment but if the genotypic difference is constitutive, its sensitivity to environmental conditions will be similar for the compared genotypes. In a previous paper (Ferrante et al., 2017), we compared the performance of Soissons (a contemporary cultivar) and Anza (a traditional cultivar) in a very wide range of growing conditions. In that study, we evidenced that Soissons had higher yield potential than Anza (averaging across years c. 7.4 and 5.8 Mg ha⁻¹, respectively) mainly due to its higher number of grains per m² (c.19,100 vs 14,700 grains m⁻², respectively). This advantage was related to its overall higher fruiting efficiency which was the basis for higher spike fertility (c. 150 and vs 110 grain g_{spike}⁻¹; see schematic representation in Fig. 1, left panel). In this paper, we tested the hypotheses that (i) Soissons possess a higher spike fertility (resulting in more fertile florets per spike) than Anza due to a faster rate of development of its floret primordia that results in an increased floret survival after the number of primordia initiated would be similar; and (ii) that these genotypic differences in floret developmental patterns would be constitutive; i.e. the sensitivity to changes in growing conditions would be similar for both genotypes (Fig.1, right panel). Thus, we compared the differences in floret developmental rates between the traditional cultivar Anza and the contemporary wheat Soissons under potential growing conditions (i.e. high nitrogen availability and natural photoperiod) and determined the sensitivity of these

floret developmental traits to (i) a shortage in N availability reducing the growth of the plants, and (ii) an extension of photoperiod during the stem elongation phase, reducing the duration of the phase when floret primordia development takes place.

2. Materials and methods

Four experiments were carried out during 2010–11 and 2011–12 growing seasons in Lleida, NE Spain (lat. 41°37'N, long. 0°35'E, altitude 180 m). In each of the two seasons (2010–11 and 2011–12) there were two independent experiments, each comparing two cultivars of contrasting yield potential under either potential conditions (i.e. fully fertilised and irrigated) or a yield reducing condition. A control vs extended photoperiod experiment and a control vs low N experiment was conducted each year. Since results were very similar in each year, they were averaged across years for the P (photoperiod) and N (nitrogen) experiments, which are known hereafter only by these names (Table 1). Therefore, to test the sensitivity of spike fertility of the two contrasting cultivars we subjected them to either (i) a change in photoperiod (a signal known to affect the length of the phase of floret development), or (ii) contrasting N availability (a resource known to affect crop growth in that phase).

Soils in all cases were fertilised with phosphorus (20 KgP ha⁻¹) and in the experiments carried out in large containers micronutrients were also applied. All experiments were irrigated when needed to avoid any water stress and diseases, insects and weeds were prevented or controlled. Thus, for the treatments of natural photoperiod - and highly fertilised condition plants were grown under yield potential conditions, and the extended photoperiod and low N treatments in each case allowed to test the sensitivity of spike fertility of the two contrasting cultivars to a signal accelerating development during pre-anthesis spike growth (P experiments) and to a limitation of a growing resource (N experiments).

The two cultivars of contrasting yield potential were Anza (a traditional, though semi-dwarf, spring cultivar released in Spain in early

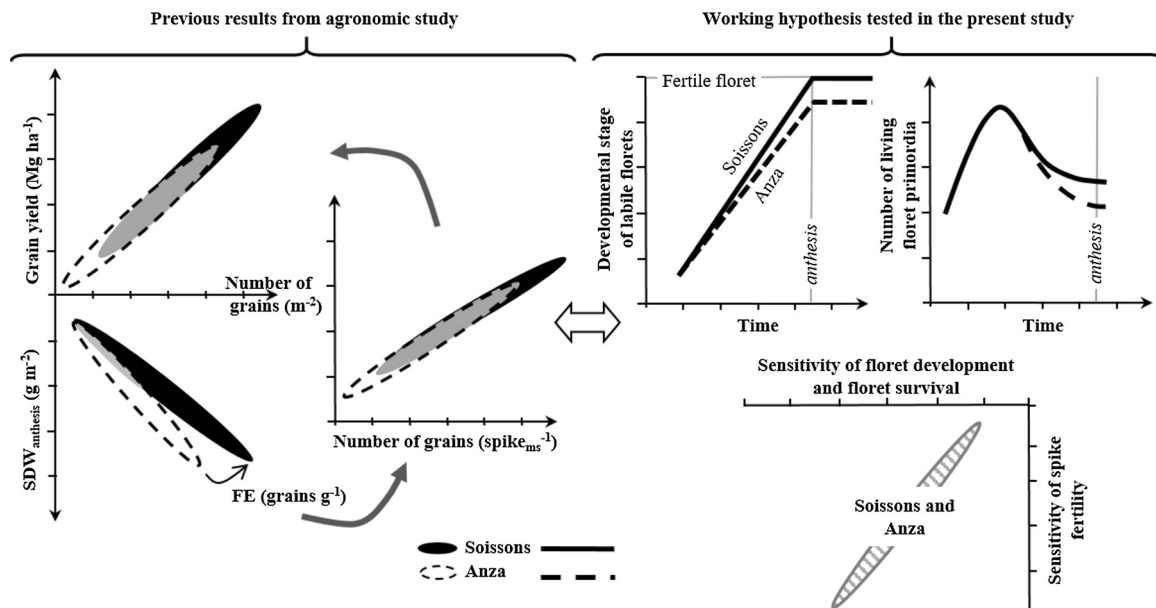


Fig. 1. Left: schematic diagram representing main results of the agronomic study comparing the performance of Soissons (a high yielding cultivar) and Anza (a traditional cultivar) in a very wide range of growing conditions (for data supporting the schemes please see Ferrante et al., 2017). The yield advantage of Soissons was mainly related to its higher efficiency to convert into grains the resources allocated to the juvenile spikes before anthesis (fruiting efficiency; the ratio between number of grains and spike dry weight [SDW] at anthesis) which increased the spike fertility of the contemporary cultivar. Right: hypothetical differences in floret developmental rates (only schematised the development of labile florets, responsible for the differences in spike fertility) and in dynamics of floret primordia initiation/degeneration (determining the final number of fertile florets), and the hypothetical similitude in sensitivity to environmental changes of fertile florets and its developmental determinants between the two cultivars that may explain the agronomic responses considering that the differences in spike fertility were constitutive.

Table 1

Experimental details (including sowing dates and rates and amount of N available in the top 1 m at sowing) for the two studies aimed to compare two contrasting cultivars (Anza, of relatively low yield potential; and Soissons, of higher yielding potential) in factorial combination with either daylength (during DC31-65) in field conditions (Experiments 1 and 2); or N availability in outdoors large containers (Experiments 3 and 4). Both studies were carried out at Lleida (NE Spain) in two growing seasons. Bold type indicates treatments within an experiment. In all experiments Anza and Soissons were also treatments combined with either photoperiod or N availability (not mentioned in the table as they were used in all cases, not establishing differences between experiments).

Experiment	Experimental design and approaches	Sowing date and density	Soil N at sowing (kgN ha ⁻¹)	Experimental treatments		Image of the experimental approach
				N applied ^a (kgN ha ⁻¹)	Daylength ^b	
1	Blocks completely randomised (3 replicates)	16 Nov. 10 320 plants m ⁻²	150	50 _{DC21}	Extended _{DC31-65} Natural Extended _{DC31-65}	
2	Field plots with photoperiod control	14 Nov. 11 290 plants m ⁻²	165	35 _{DC21}		
3	Completely randomised (3 replicates)	12 Nov. 10 250 plants m ⁻²	20	30 _{DC21} 60 _{DC21} + 60 _{DC23} + 60 _{DC31}	Natural	
4	Large containers outdoors ^c	11 Nov. 11 320 plants m ⁻²	20	30 _{DC21} 60 _{DC21} + 60 _{DC23} + 60 _{DC31}		

^a DC 2.1; Zadoks et al. (1974). Fertiliser was applied splitting the dose in two or three equal applications at the onset of tillering (DC 2.1), at mid-tillering (DC 2.3) and the onset of stem elongation (DC 3.1).

^b Daylength was extended (relatively long days) only during the stem elongation phase from DC31 to DC65 in the plots assigned this treatment and natural (relatively short days) throughout the rest of the growing season in these treatments, and throughout the whole growing season in the "controls" in experiments 1 and 2 and in all treatments in experiments 3 and 4.

^c Seeds were manually placed at precise regular intervals, to maximize uniformity of the stand, on masking tape, then these 1 m linear strips were covered with tissue paper and placed in the rows of each experimental unit. The consequence of the delicate handling for sowing was the achievement of experimental units with almost perfectly uniform microcrops (see details in Ferrante et al., 2010).

Table 2

Time from sowing to anthesis (A), and global radiation, average temperature and photothermal quotient (PTQ) from terminal spikelet to anthesis (DC31-65). For experimental detail refer to Table 1.

Experimental design and approaches	Experiment	Experimental treatments			Time to A Global radiation (days)	Average temp (°C)	PTQ (Mj m ⁻² d ⁻¹ °C ⁻¹)
		N applied ^a (kgN ha ⁻¹)	Daylength ^b	Cultivar			
Randomised block design (3 replicates) Field plots with photoperiod control	1	50 _{DC21}	Natural	Anza	161.0 ± 0.0	19.8 ± 4.5	16.1 ± 2.4
				Soissons	168.0 ± 0.0	20.7 ± 4.6	16.7 ± 1.9
			Extended	Anza	157.0 ± 0.0	20.7 ± 3.6	16.2 ± 2.6
				Soissons	165.0 ± 0.0	20.5 ± 4.7	16.7 ± 2.0
	2	35 _{DC21}	Natural	Anza	166.0 ± 0.0	19.3 ± 5.2	13.3 ± 2.3
				Soissons	176.0 ± 0.0	20.4 ± 5.3	13.9 ± 2.3
			Extended	Anza	163.0 ± 0.0	19.3 ± 5.1	13.0 ± 1.9
				Soissons	170.3 ± 1.2	19.8 ± 5.2	13.6 ± 2.2
Completely randomised design (3 replicates) Large containers outdoors	3	30 _{DC21}	Natural	Anza	166.0 ± 0.0	20.1 ± 5.0	17.0 ± 2.1
				Soissons	168.0 ± 0.0	20.9 ± 5.0	16.8 ± 2.0
				Anza	166.0 ± 0.0	20.1 ± 5.0	17.0 ± 2.1
				Soissons	168.0 ± 0.0	20.9 ± 5.0	16.8 ± 2.0
	4	60 _{DC21} + 60 _{DC23} + 60 _{DC31}	Natural	Anza	165.0 ± 0.0	19.2 ± 5.2	13.4 ± 2.3
				Soissons	173.0 ± 0.0	20.6 ± 5.5	14.0 ± 2.3
			60 _{DC21} + 60 _{DC23} + 60 _{DC31}	Anza	165.0 ± 0.0	19.2 ± 5.2	13.4 ± 2.3
				Soissons	173.7 ± 0.6	20.5 ± 5.4	14.1 ± 2.4

^a DC 2.1; Zadoks et al. (1974). Fertiliser was applied splitting the dose in two or three equal applications at the onset of tillering (DC 2.1), at mid-tillering (DC 2.3) and the onset of stem elongation (DC 3.1).

^b Daylength was extended (relatively long days) only during the stem elongation phase from DC31 to DC65 in the plots assigned this treatment and natural (relatively short days) throughout the rest of the growing season in these treatments, and throughout the whole growing season in the "controls" in experiments 1 and 2 and in all treatments in experiments 3 and 4.

1970's) and Soissons (a contemporary, higher-yielding, winter cultivar released in Spain in 1990). At the time we started the studies including the experiments reported in this paper, Anza was the most popular choice by most conservative farmers (those averse to risk) and Soissons represented a cultivar popularly chosen by more risky farmers aiming to maximise yields (e.g. Abeledo et al., 2014, 2008, and referenced quoted therein). But both were within the most popular cultivars grown

in the region. Indeed, they were used as controls in yield comparative trials conducted by the Group for the Evaluation of the New Cereals Varieties in Spain (GENVCE) to test the performance of newer cultivars (Abeledo et al., 2014). In our growing conditions, winter and spring cereals are sown together in mid-late November (as we did in this study, see Table 1) and those crops emerge in early- or mid-December when daylength is within the shortest of the year and temperatures over

winter are low (as shown in Fig. 1 in Ferrante et al., 2017). Under these conditions, unlike what would be the case in traditional winter wheat growing regions, there is very small differences in phenology between winter and spring wheats when they are sown simultaneously in late fall. In these experiments in particular, Anza flowered only few days earlier than Soissons (Table 2), as it happened in many previous studies (e.g. Cartelle et al., 2006). Consequently, the solar radiation levels, temperatures and photothermal quotients during the period when florets develop (i.e. from terminal spikelet to anthesis) were virtually the same for both cultivars across all conditions (Table 2).

The contrasting soil N availabilities were imposed in the microcrops (10 rows, 0.10 m apart) grown in large containers. To maximise stand density-uniformity, which is indispensable for this type of approach, these microcrops were sown with a dedicated procedure maximising uniformity of the stand (for details on experimental setup, sowing procedure and appearance of the microcrops, please see Fig. 1 in Ferrante et al., 2010). As the N content in the soil of the containers was very low, the two contrasting N availabilities were obtained by a very light fertilisation to reach a starting N availability equivalent to only 50 kgN ha⁻¹, while the high N availability treatment was heavily fertilised to reach a N availability equivalent to 200 kgN ha⁻¹ (Table 1).

Regarding photoperiod, treatments consisted of a control with natural photoperiod (short) and an extended (long) photoperiod only during stem elongation (i.e. photoperiod was natural from sowing to the onset of stem elongation and during grain filling, but between jointing and anthesis it was extended artificially). In this case, photoperiod during this period was extended to 24 h with low-intensity incandescent lamps installed over the field plots assigned to this treatment (Table 1).

In each experimental unit, one plant was selected at random for sampling once or twice a week, the actual frequency depending on temperature (i.e. three plants per treatment combination of cultivar x photoperiod in P experiments, and cultivar x N in N experiments). Their main shoot was dissected under a binocular microscope (Leica MZ 7.5, Leica Microscopy System Ltd, Heerburg, Switzerland) to determine double ridge formation stage, as well as, terminal spikelet initiation (Kirby and Appleyard, 1984). From terminal spikelet initiation to few days after anthesis this sampling was intensified to twice or thrice a week, again depending on temperature, and the spike of the main shoot was dissected (again under a binocular microscopy) to count the total number of floret primordia in basal (third-fifth spikelet from the bottom), central (middle spikelets) and apical (third-fifth spikelet from the top) spikelets throughout the spike (Fig. 2).

Furthermore, the developmental stage of each floret (floral score) was determined, following the scale proposed by Waddington et al. (1983), as illustrated in Ferrante et al., 2013a; see Fig. 1 therein). Floret primordia within spikelets were counted and numbered from F1 (floret primordium closest to the rachis) to Fn (the most distal floret primordium of the particular spikelet).

To determine the distribution of fertile florets within the spikes (i.e. “mapping” fertile florets in the spikes) we randomly selected and sampled 10 (2010–11) and 5 (2011–12) main shoot spikes per experimental unit (i.e. 30 or 15 spikes per each individual treatment combination). Then, we separated each spikelet from one side of the rachis and open them individually counting fertile florets in each spikelet (Fig. 2). Later, we counted fertile florets in all other spikelets to have the final value of fertile florets per spike. As there is some degree of variation in developmental stages of individual florets between and within spikelets, we considered fertile florets in that sample to be any floret that were or had been already at the stage W10 (Waddington et al., 1983) or that were immediately before W10 (i.e. when the stigmatic branches were curved with green anthers), to take into account the florets of the most delayed positions of the spike that will become fertile florets soon after the sampling of anthesis.

ANOVA was performed for each trait analysed using the General Linear Model (GLM) procedure of SAS (2002), considering the

particular factorial combination treatments and experimental design in each experiment. Regression analyses were carried out on the means across replicates.

3. Results

3.1. Physiological bases for spike fertility differences between cultivars

The difference between Soissons and Anza in number of grains per spike was due to their consistent differences in the number of fertile florets: Soissons had significantly more fertile florets per spike than Anza under natural photoperiod and high N availability (i.e. potential conditions; 46.7 vs 40.0; $P = 0.003$; Fig. 3, left panel). Under these conditions, grain set, the ratio between fertile florets per spike and grains per spike expressed as percentage, was similar for both genotypes (averaging across experiments 89.6 and 87.2 % in Soissons and Anza, respectively). The analysis of the positions of the spike where these genotypic differences under potential conditions of natural photoperiod and 200 kgN ha⁻¹ were established evidenced that in all experiments the higher fertility of Soissons than Anza was concentrated in the central and apical spikelets (Fig. 3, right panels). Thus, we focused of the developmental features of florets in central and apical spikelets.

The two floret primordia closest to the rachis (florets 1 and 2) developed at very similar rates in both cultivars until reaching the stage of fertile florets in both central and apical spikelets (Fig. 4). The same was true for floret 3 in central spikelets (Fig. 4). But floret 3 of apical spikelets did exhibit a difference between the two cultivars that was consistent across experiments. While it developed normally until reaching the stage of W10 in some plants it did not reach the stage of W10 in others. Consequently, the proportion of plants having a third fertile floret in the apical spikelets was larger in Soissons than in Anza (Fig. 4). A very similar scenario was shown by floret 4 in central spikelets (Fig. 4). All other floret primordia within either apical or central spikelets were never developed enough to reach the stage of fertile floret in any of the cultivars. But even in the case of these primordia (i.e. florets 5, 6 and 7 of central spikelets and 4, 5, 6 and 7 of apical spikelets), they reached more advanced stages of development in Soissons than in Anza (Fig. 4).

Considering together the rates of development of all floret primordia, we analysed the dynamics of the number of living floret primordia (i.e. initiated floret primordia at each sampling that continued developing normally towards more advanced developmental stages) from once terminal spikelet initiation was detected until few days after anthesis (Fig. 5). It can be noticed that the difference in number of florets that reached the stage of fertile florets per spikelet was small (see in Fig. 3, right panels, that in most central and apical spikelets Soissons had only less than 0.5 more fertile florets than Anza), but collectively produced a major difference in spike fertility (Fig. 3, left panel). That difference in final number of fertile florets in central and apical spikelets was mainly because Soissons reduced the rate of floret mortality compared with Anza (Fig. 5).

3.2. Spike fertility sensitivity to daylength and soil N availability

The magnitude of the sensitivity of spike fertility was rather small with the manipulation of photoperiod and quite substantial with the manipulation of N availability; but similar for both cultivars (Fig. 6). The F-ratio for the interaction cultivar x photoperiod was not significant (0.12, $P = 0.73$ in the first growing season and 2.95, $P = 0.12$ in the second season); as well as for the interaction cultivar x N (0.01, $P = 0.96$ in 2010–11 and 0.22, $P = 0.65$ in 2011–12).

Lengthening the photoperiod during stem elongation accelerated anthesis by c. 100 °C d in both cultivars (Ferrante et al., 2017) which resulted in a small reduction in the number of fertile florets per spike, determining in turn a small reduction in grains per spike (Fig. 6, open symbols). When the crop was grown under a very limited availability of

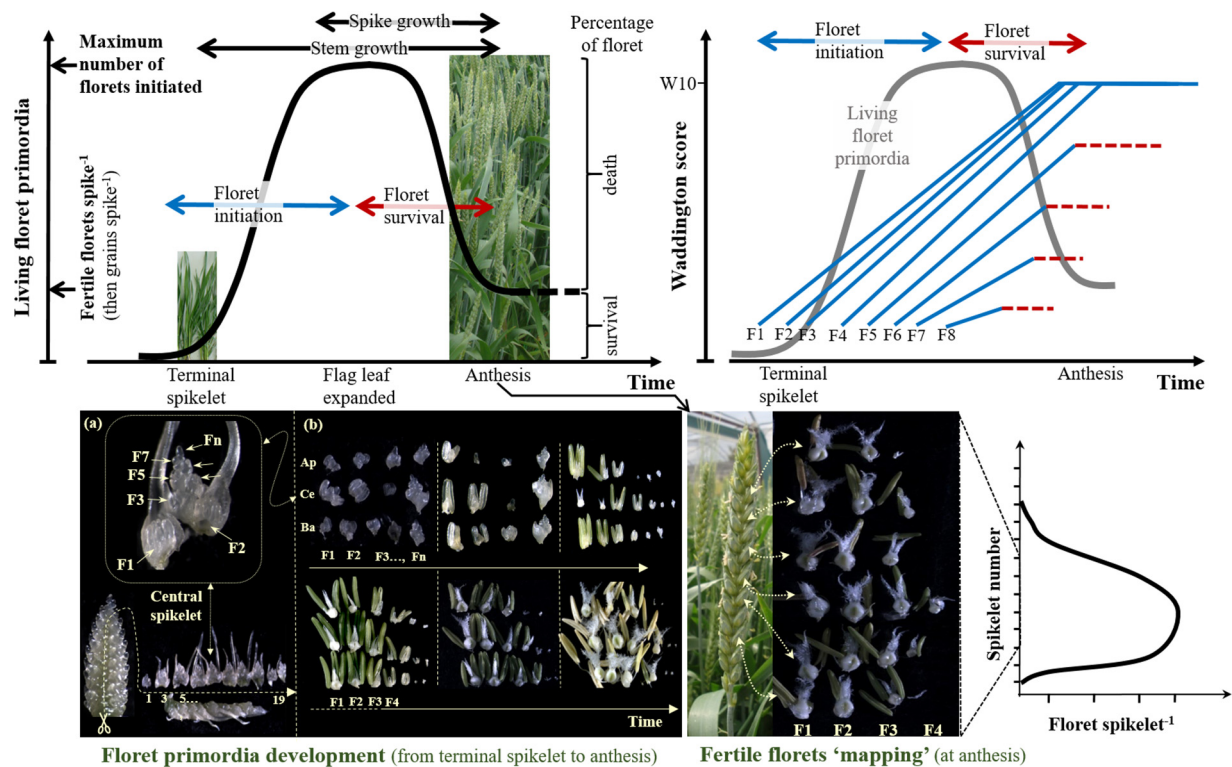


Fig. 2. Illustration of the dynamics of (i) living floret primordia (floret initiation followed by floret survival; solid line), determining the final number of fertile florets per spike (dotted line) throughout the wheat late reproductive phase of stem elongation, from terminal spikelet to anthesis (top left panel); and (ii) individual floret development within a spikelet: floret primordia are initiated sequentially from F1 to Fn, all of them contributing to floret initiation; the most proximal florets develop normally until the stage of fertile floret (W10) while more distal florets stop developing and die in the reverse order in which they were initiated determining floret mortality (top right panel). Bottom left box showing floret primordia development: (a) spike dissected highlighting in detail a particular spikelet showing all floret primordia from the most proximal (F1) to the most distal (Fn) respect to the rachis. (b) photos showing a wide range of different developmental stages (Ferrante et al., 2013a; Waddington et al., 1983) from terminal spikelet to anthesis in all floret primordia from F1 to Fn in apical (Ap), central (Ce) and basal (Ba) spikelets. Bottom right box: a spike at anthesis, illustrating the mapping of fertile florets in the different spikelets of the spike. This figure is available in colour at FCR online.

N, the penalty on the number of grains per spike was rather large, due to the reductions in the number of fertile florets per spike (Fig. 6, closed symbols) and very similar for both cultivars. When growing under lower-yielding conditions (longer photoperiod or reduced availability of N), the penalties were focused in the central and basal spikelets (and naturally the magnitude of the difference was very small for daylength extension and rather large for the strongly reduced N availability). The number of fertile florets per apical spikelet was not, or only marginally, reduced (Fig. 6, bottom panels).

Sensitivity of the number of fertile florets averaging all three spikelet positions analysed was due to the sensitivity of both the maximum number of florets initiated as well as that of the survival rate of those primordia to become fertile florets (Fig. 7). Naturally the effect of photoperiod extension was small for both components of fertile florets per spike and that of N availability was rather major and similar for both cultivars (Fig. 7). Although the effects on spike fertility were through affecting both components, the relative contribution of floret survival was higher than that of the maximum number of florets initiated in response to N, the treatment having a major effect on spike fertility (Fig. 7).

Thus focusing on the responses to N availability and on the central and basal spikelets (where the responses were clear; Fig. 6, bottom panels) it was evident that both cultivars responded similarly to a strong decrease in N availability with respect to the potential yielding condition. In all cases (both cultivars, central and basal spikelets), there was a depression in the total number of floret primordia initiated followed by a further reduction in the survival of these initiated primordia (Fig. 8). The reduction of both the number of primordia initiated and the survival of floret primordia to produce fertile florets may well be the

consequence of the overall deceleration of floret development under N deficiency conditions that was apparent in most floret primordia of both cultivars and regardless of the spikelet position considered, though it was more clear in more labile florets (i.e. floret primordia that depending on the conditions survive or die, being mainly responsible for differences in spike fertility: most frequently F3 and F4 positions with respect to the rachis; Fig. 9). Even when developing slower under low N availability, florets 1 and 2 (the two most proximal to the rachis) reached the stage of fertile florets and therefore the N stress did not produce a penalty in the final fertility of these florets (Fig. 9). The effect became relevant in establishing differences in the number of fertile florets for primordia developing in positions 3 and 4 from the rachis in which the slower development of these florets under low N determined that most/all these florets did not develop normally to reach the stage of fertile floret (they died at some stages of development). While under high N availability many of these florets progressed normally to reach the stage of fertile floret (Fig. 9). Even for the floret positions that died in all cases (in both cultivars and in central and basal spikelets, e.g. florets 5–7), it was clear that the development was much faster under high than under low N availability (Fig. 9). Thus, if we calculate the average stage of development reached by all floret primordia in each of the treatments it was clear that a reduction in N availability resulted in final stages of development of only c. 50 % of the equivalent stage at high N availability (Fig. 10).

4. Discussion

The number of grains of the main shoot spikes was a major determinant of differences in yield potential between Soissons and Anza

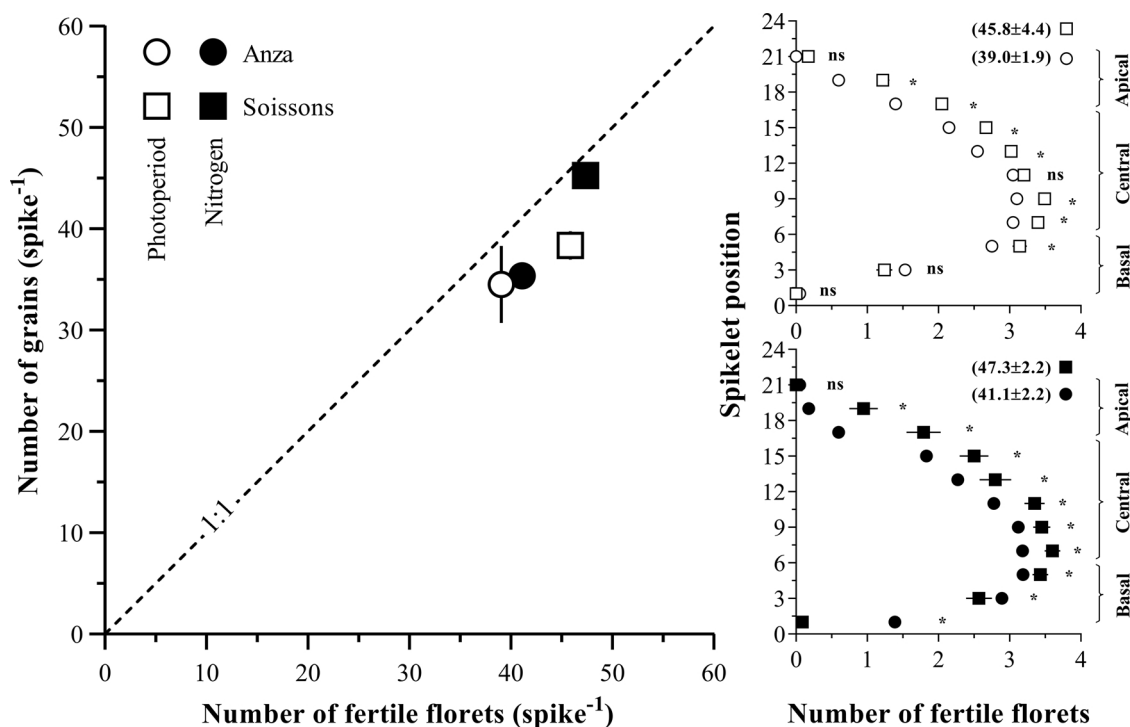


Fig. 3. Left: relationship between number of grains at maturity and number of fertile florets at anthesis in main shoot spikes for Anza and Soissons in the potential yielding condition of natural photoperiod (P experiments, open symbols) and 200 KgN ha⁻¹ (N experiments, closed symbols). Right: “mapping” of fertile florets at anthesis, detailing the distribution of florets in the abscissa of the right panel; i.e. number of fertile florets each spikelet position (from spikelet 1, at the base of the spike, to the terminal spikelet). Segments in each symbol stand for the standard error of the means (which was smaller than the body of the symbol if not visible). On the right of the panels representing the “mapping” of fertile florets it is indicated which spikelets are represented by the basal, central and apical spikelets dissected for determining the development of floret primordia). Asterisks represent that the differences between fertile florets was equal or higher than the standard deviations. The opposite case was represented by ns. Values between brackets are means $\pm$ standard deviations.

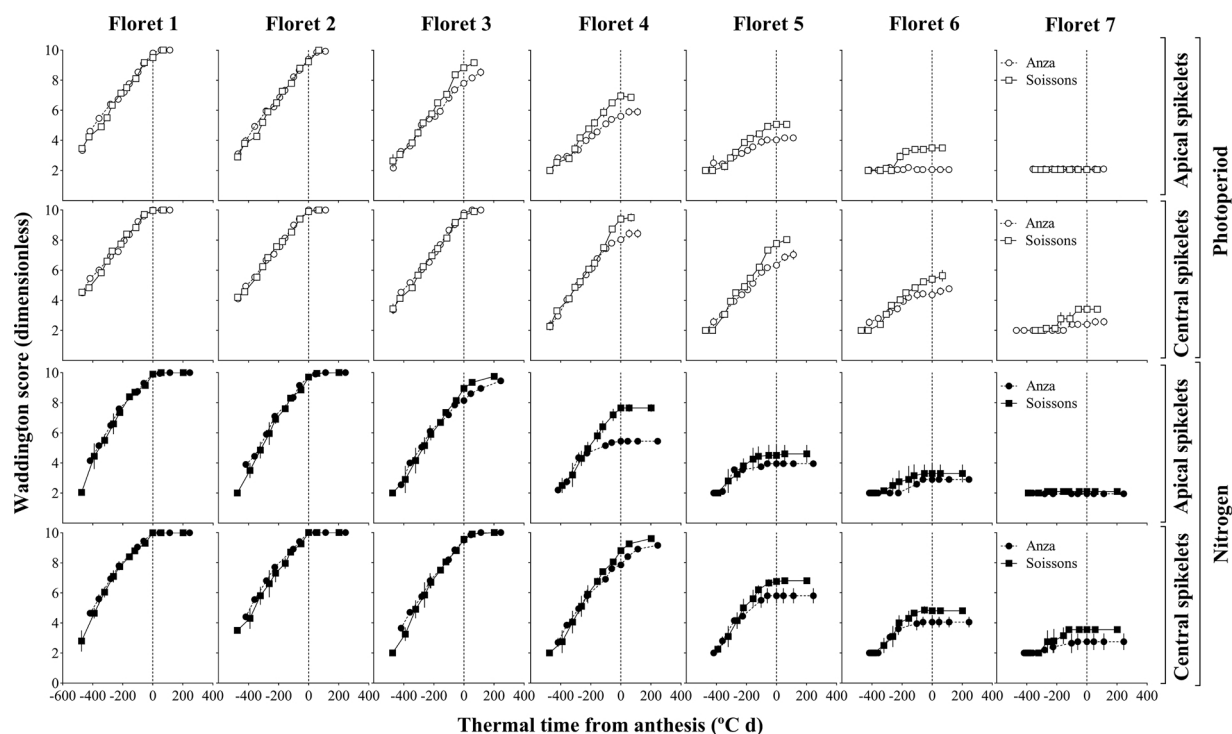


Fig. 4. Dynamics of spikelet floret development from floret 1 (F1, floret primordium closest to the rachis) to floret 7 (F7, floret primordium most distal to the rachis) in each of the two spikelet categories considered of the main-shoot through thermal time from anthesis (negative values represent the period before anthesis) in the potential yielding condition of natural photoperiod (P experiments, open symbols) and 200 KgN ha⁻¹ (N experiments, closed symbols). Each data-point is the average of all replicates across two growing seasons and within each replicate the value was the average of 10 (2010-11) and 5 plants (2011-12), bars represent the standard error of the means (which, if not visible, was smaller than the body of the symbol).

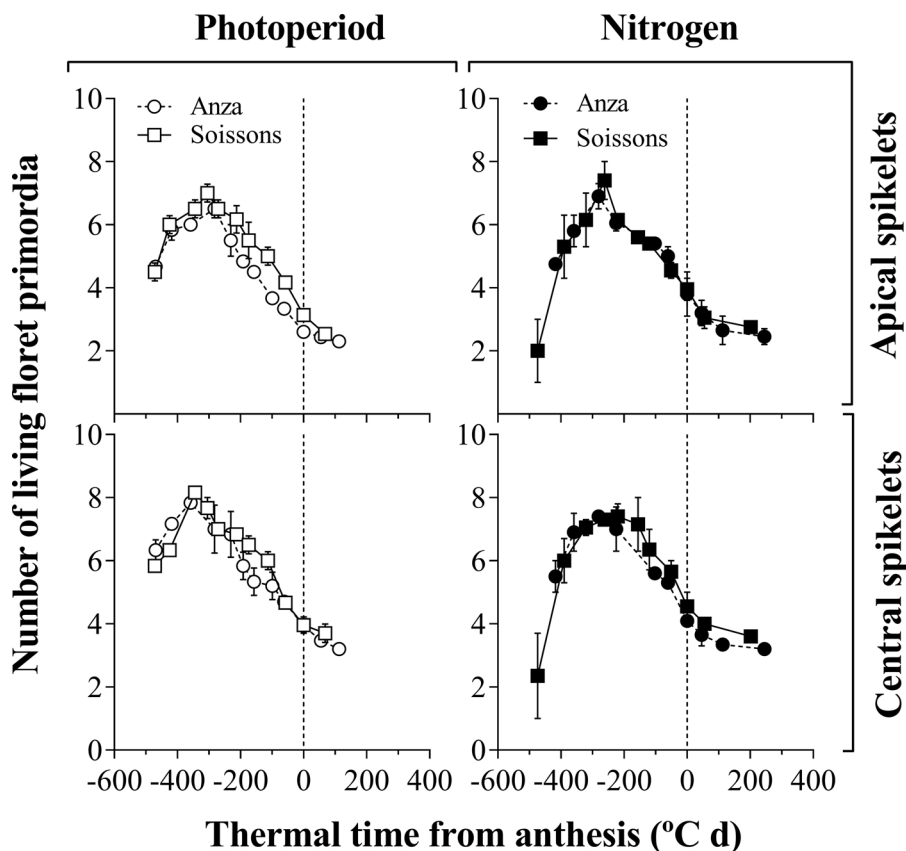


Fig. 5. Dynamics of living floret primordia in central and apical spikelets of the main-shoot through thermal time from anthesis (negative values represent the period before anthesis) in the potential yielding condition of natural photoperiod (P experiments, open symbols) and 200 KgN ha⁻¹ (N experiments, closed symbols). Each data-point is the average of all replicates across two growing seasons and within each replicate the value was the average of 10 (2010-11) and 5 plants (2011-12), bars represent the standard error of the means (not visible in some cases as it was smaller than the body of the symbol).

(Ferrante et al., 2017), which is in line with a more general analysis concluding that changes in grains per m² are mainly dependent on changes in spike fertility (Slafer et al., 2014). For that reason, this paper focuses on the mechanistic bases of differences in spike fertility; which would depend upon the differences in dynamics of floret initiation and mortality within the growing spike before anthesis (Ferrante et al., 2013a, 2010; González-Navarro et al., 2015; Guo et al., 2016).

In this study, we showed for the first time that the difference in spike fertility between a traditional (low-yield potential) and contemporary (high yield potential) cultivar of wheat were due to their differential rates of development of some labile florets (in both central and apical spikelets). This acceleration of floret primordia development allowed some labile florets to reach the stage of fertile florets whilst the slower development of these florets in the traditional cultivar implied that they failed to complete their development. This is in line with the evidences that the introgression of Rht alleles increased spike fertility through allowing relatively distal florets, which die in tall genotypes, to maintain a normal development increasing floret fertility (Fischer and Stockman, 1986; Miralles et al., 1998). In addition, this behaviour was clear not only in these labile florets but also in primordia of more distal florets that died in all cases but reached later stages of development in the contemporary than in the traditional cultivar. Due to this faster rate of development across a range of floret primordia, the contemporary cultivar initiated more florets primordia as well as reduced the rate of mortality of the initiated floret primordia. Although both components of the number of fertile florets per spike were improved when comparing the contemporary vs the traditional cultivar, it was clear that the survival of initiated florets was much more relevant than the initiation of floret primordia to establish the improved spike fertility. This is in line with the hypothesis that the plasticity of a given component could be expected to depend mainly on plasticity of the component representing a more significant cost for the plant to maximise its reproductive output (Sadras and Slafer, 2012). The metabolic cost required to initiate floret primordia would be negligible compared with

that required to maintain floret growth to the stage of fertile floret (Ferrante et al., 2013b).

The sensitivity of the number of fertile florets exposed to contrasted N availability and different photoperiod conditions evidenced that the improved spike fertility in the higher yielding cultivar was constitutive: this improvement did not bring about a larger sensitivity to stresses indicating that the spike fertility advantage would be maintained across a wide range of growing conditions. Thus genetic gains in spike fertility would be not only relevant for high-yielding conditions. This provides a mechanistic foundation for evidences in the literature that improving yield potential would bring about constitutive improvements under stressed conditions as well (e.g. Araus et al., 2008; Cartelle et al., 2006; Richards, 2000).

Reinforcing the results, when comparing the two contrasting genotypes under potential conditions, it was again clear that the final effect of N availability for plant growth on spike fertility was mainly mediated through affecting the mortality of floret primordia with a much smaller effect on the maximum number of florets initiated. This confirms previous results on durum wheat (Ferrante et al., 2013a, 2010) and barley (Arisnabarreta and Miralles, 2010).

In the present study, an effect of N stress on the final level of spike fertility was mediated through the effect on the rate of floret primordia development as it was the case for establishing the genotypic effect under potential conditions. This means that the developmental rate and in particular their survival once initiated of individual floret primordia seems to depend on the availability of resources, which is consistent with the hypothesis proposed by Dreccer et al. (2014) for the co-ordination between floret developmental rates (that would be accelerated relative to the phasic developmental rate to improve floret fertility) and resources allocated to the growing spike before anthesis. Therefore, when there are more resources available labile floret primordia develop faster than when resources are more limited, allowing these labile primordia to reach the stage of fertile floret (W10) through reducing the mortality of these florets and increasing spike fertility

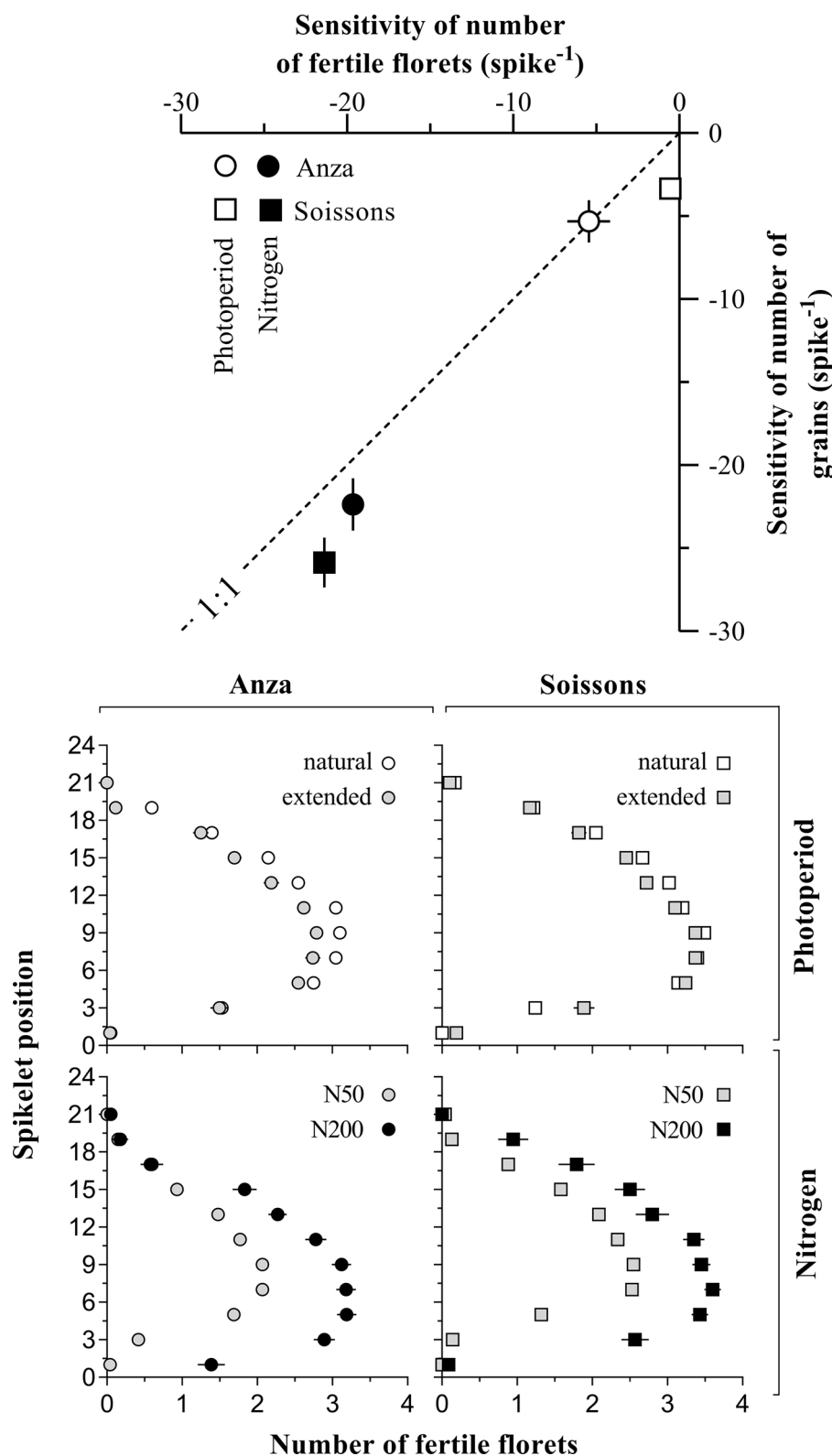


Fig. 6. Top: relationship between the sensitivity to either an extension of photoperiod during stem elongation (P experiments) or to a low N availability (N experiments) of the number of grains at maturity and of the number of fertile florets at anthesis in main shoot spikes for Anza and Soissons [sensitivity was the difference between these traits in either the extended vs the natural photoperiod or the low vs the high N availability]. Bottom: “mapping” of fertile florets at anthesis [number of fertile florets in each spikelet position in the contrasting treatments for each of the two cultivars]. Segments in each symbol stand for the standard error of the means (not visible in some cases as it was smaller than the body of the symbol).

(Ferrante et al., 2013a, 2010). This would also align with the idea that increasing the concentration of water-soluble carbohydrates in stems, at the time spike fertility is being determined, tended to reduce the number of grains growing after anthesis (Rebetzke et al., 2008), though the accumulation of water-soluble carbohydrates may not produce this effect if accompanied by an increase improvements in photosynthetic activity, as it seems possible at least in some isogenic lines for water-

soluble carbohydrates accumulation in stems (Dreccer et al., 2014).

Finally, most floret survival studies mentioned the importance of C flux to the growing spike when considering environmental factors affecting plant growth (e.g. Ferrante et al., 2013a) or genes affecting C partitioning (e.g. Miralles et al., 1998). However, in the present study we did not find relevant differences between Soissons and Anza in spike dry weight at anthesis. Therefore, differences in floret

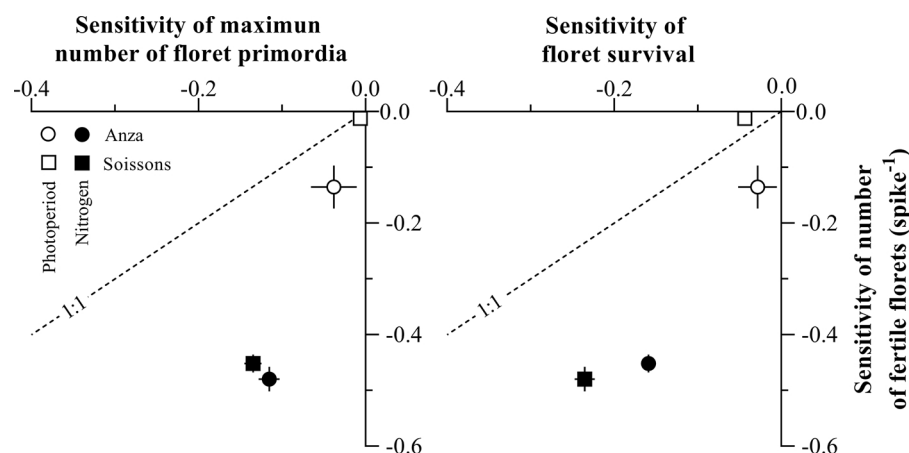


Fig. 7. Relationship between the sensitivity to either an extension of photoperiod during stem elongation (P experiments) or to a low N availability (N experiments) of fertile florets and the maximum number of floret primordia (left) or floret survival (right) for Anza and Soissons (sensitivity was the difference between these traits in either the extended vs the natural photoperiod as a proportion of the value reached in natural photoperiod or the low vs the high N availability as a proportion of the latter). Each data-point is the average of the three spikelet positions analysed.

development resulting in differences in spike fertility reflected the genotypic differences in fruiting efficiency (Slafer et al., 2015). In turn, the fact that the sensitivity of floret development and spike fertility to either to faster development, due to exposure to longer days, or to less available soil N were similar for both cultivars reveals that their differential floret development and spike fertility were constitutive and determines an overall higher yield of the high yield potential genotype across a wide range of conditions (Ferrante et al., 2017).

We conclude that the difference in yield potential associated to parallel differences in spike fertility reflected a differential rate of floret development of the genotypes. Thus, the higher yielding cultivar exhibited florets developing faster than the traditional cultivar and that difference in rate of development of the florets was responsible for an improved survival of florets initiated which brought about differences in fruiting efficiency. Furthermore, these differential rates of floret development seemed to be constitutive as the sensitivity of spike fertility to either extended photoperiod or reduced soil N availability was similar for both genotypes (and therefore their differences in spike

fertility maintained across contrasting environments).

CRediT authorship contribution statement

Ariel Ferrante: Methodology, Investigation, Formal analysis, Visualization, Writing - original draft. **Roxana Savin:** Supervision, Methodology, Writing - review & editing. **Gustavo A. Slafer:** Conceptualization, Supervision, Methodology, Formal analysis, Visualization, Funding acquisition, Project administration, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

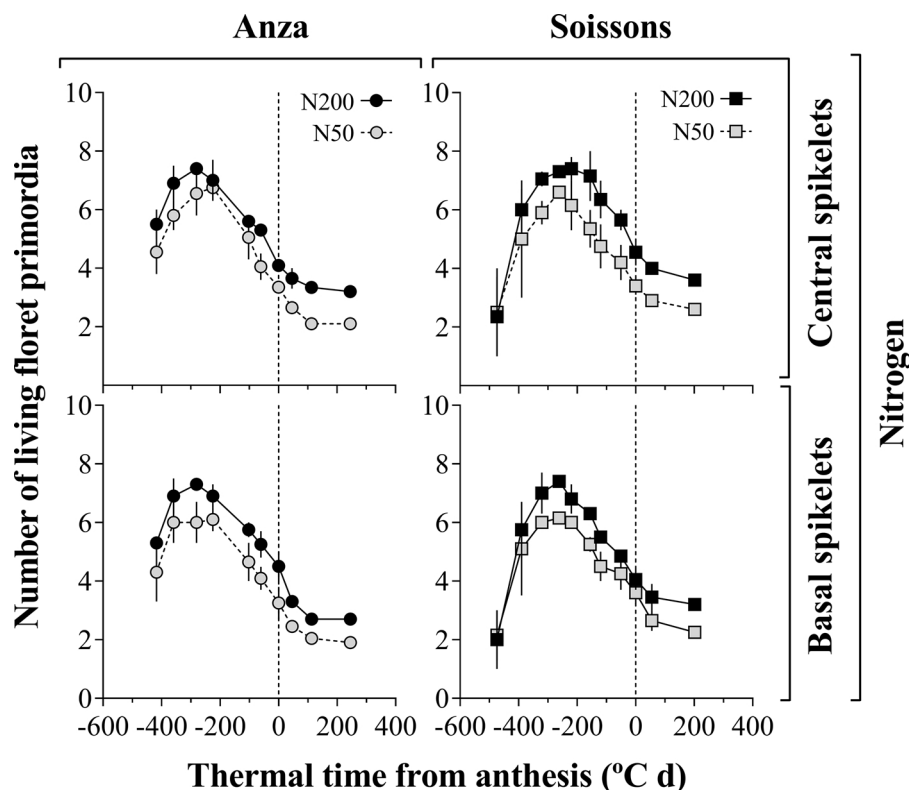


Fig. 8. Dynamics of living floret primordia in each of the two spikelet categories considered of the main-shoot through thermal time from anthesis (negative values represent the period before anthesis) in the N experiments for Anza and Soissons. Grey and black symbols correspond to N50 (50 KgN ha⁻¹) and N200 (200 KgN ha⁻¹). Each data-point is the average of all replicates across two growing seasons and within each replicate the value was the average of 10 (2010-11) and 5 plants (2011-12), bars represent the standard error of the means (not visible in some cases as it was smaller than the body of the symbol).

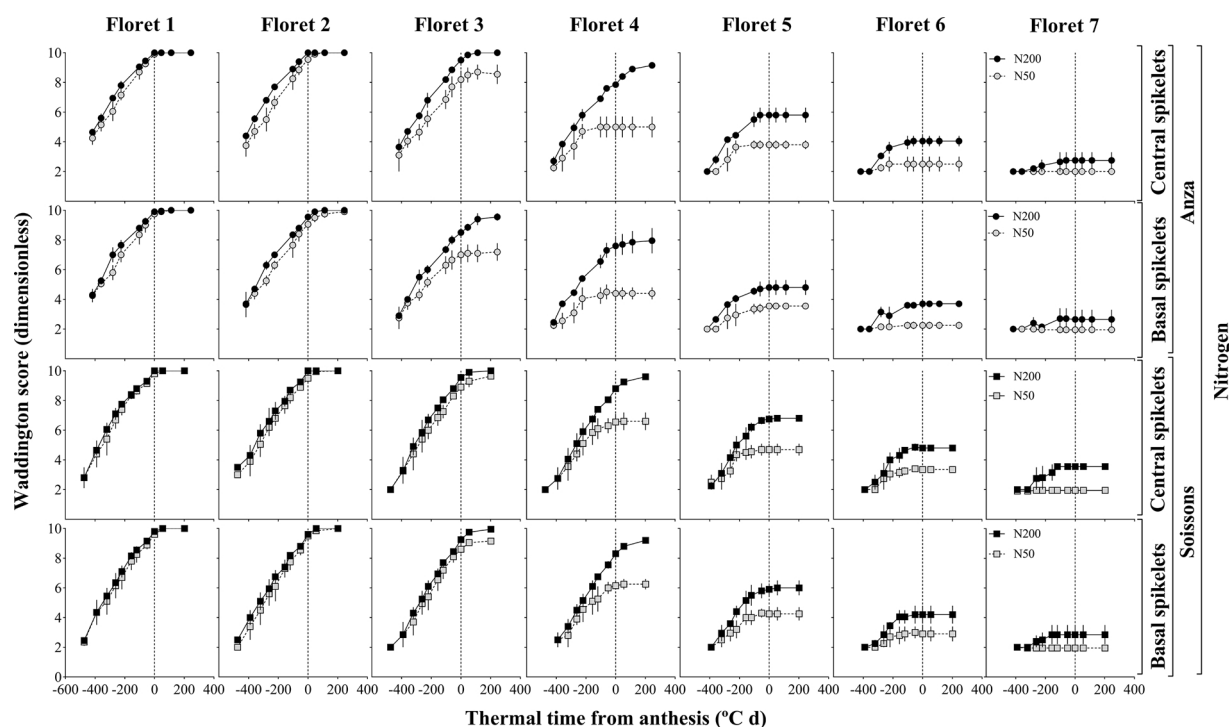


Fig. 9. Dynamics of the floret development from floret 1 (F1, floret primordium closest to the rachis) to floret 7 (F7, floret primordium most distal to the rachis) in each of the two spikelet categories considered of the main-shoot through thermal time from anthesis (negative values represent the period before anthesis) in the N experiments for Anza and Soissons. Grey and black symbols correspond to N50 (50 KgN ha⁻¹) and N200 (200 KgN ha⁻¹). Each data-point is the average of all replicates across two growing seasons and within each replicate the value was the average of 10 (2010-11) and 5 plants (2011-12), bars represent the standard error of the means (not visible in some cases as it was smaller than the body of the symbol).

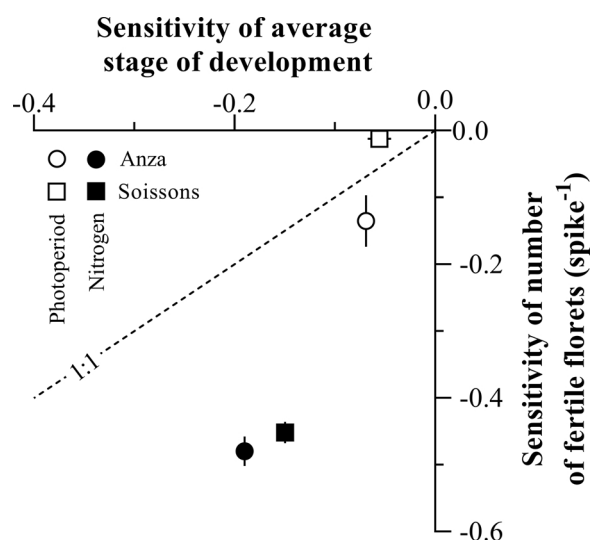


Fig. 10. Relationship between the sensitivity to either an extension of photoperiod during stem elongation (P experiments) or to a low N availability (N experiments) of the fertile florets at anthesis and of the average stage of development in all florets at anthesis in main shoot spikes for Anza and Soissons (sensitivity was the difference between these traits in either the extended vs the natural photoperiod or the low vs the high N availability). Bars represent the standard error of the means (not visible in some cases as it was smaller than the body of the symbol). Each data-point is the average of the three spikelet positions analysed.

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