

RESEARCH PAPER

Floret development of durum wheat in response to nitrogen availability

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Abstract

In Mediterranean durum wheat production, nitrogen (N) fertilization may be important to stabilize and increase yields. Wheat yield responses to N fertilization are usually related to grains per m², which in turn is the consequence of processes related to floret development (floret initiation followed by floret death/survival) during stem elongation. The literature is rather scarce in terms of the relevance of floret developmental dynamics, determining the final number of grains in general terms and in particular regarding responsiveness to N. The aim of this study was to determine whether durum wheat responses to N under different water regimes are related to the dynamics of development of floret primordia to produce fertile florets. During the 2006–2007 and 2007–2008 growing seasons, experiments with a factorial combination of two N levels (0 and 100 or 250 kg N ha⁻¹) and two levels of water availability (rainfed and irrigated) were carried out (although the water regime was only effective in the second season). The response of yield was largely a consequence of that in grain number per spike. Floret initiation was similar for both N levels in each experiment and water regime, for which the survival of a higher proportion of initiated florets was critical in the response of the crop. The diminished rate of floret abortion during the late part of stem elongation in response to N was associated with a slightly accelerated rate of floret development which allowed a higher proportion of the primordia initiated to reach the stage of fertile floret by flowering.

Key words: Floret primordia, grain number, *Triticum durum*, yield.

Introduction

Durum wheat is a major crop in the Mediterranean basin of West Asia, North Africa, and Southern Europe (Elias and Manthey, 2005). Yields of rainfed cereals in Mediterranean conditions are quite variable mainly due to erratic rainfall regimes (e.g. Austin *et al.*, 1998). Part of this variability might be reduced through a better management of nitrogen (N), as recently discussed by Abeledo *et al.* (2008). This is because water use efficiency may be greatly affected by N management, even under terminal stress conditions characteristic of Mediterranean regions (Passioura, 2002).

When analysing physiological determinants of cereal productivity, yield is commonly divided into its two major components: the number of grains that the crop sets per m² and the average weight of these grains. Although these components are frequently negatively related, the nature of

this relationship is only seldom competition among growing grains for limited assimilates (Slafer and Savin, 1994; Borrás *et al.*, 2004), as grain growth is most frequently sink limited rather than source limited (e.g. Reynolds *et al.*, 2005; Acreche and Slafer, 2006; Bingham *et al.*, 2007), even under Mediterranean conditions (e.g. Cartelle *et al.*, 2006; Acreche *et al.*, 2008). This is why yield in wheat (as well as in most grain crops) is largely determined by the number of grains per m² (Slafer *et al.*, 2006); and understanding the mechanisms controlling the determination of the number of grains per m² may be relevant (Slafer, 2003; Fischer, 2007).

The number of grains per unit land area is being formed throughout the whole pre-flowering period (Slafer and Rawson, 1994) as a consequence of a rather complex process through which structures, which might later be able

to bear grains, are first generated and then—a rather large proportion of them—degenerate. The dynamics of these structures are tillering followed by tiller death, and floret initiation followed by floret death (Kirby, 1988; Longnecker *et al.*, 1993; González *et al.*, 2005a, b; Bancal, 2009). As a consequence of the dynamics of generation/degeneration, only a rather small fraction of the total number of potential grain-bearing structures produced by the crop through the tillering and stem elongation phases actually ends up setting grains (Reynolds *et al.*, 2009).

There are many studies in the literature reporting differences in the number of grains per m² of cereals due to environmental effects as well as to genotypic differences (e.g. Egli, 1998; Slafer *et al.*, 2006). However, evidence of whether these differences among genotypes and responses to the environment are related to the processes of generation or degeneration of floret primordia is rather scarce, while results analysing effects on tillering and tiller mortality are far more common (Longnecker *et al.*, 1993; Baethgen *et al.*, 1995; Grashoff and D'Antuoro, 1997; Rodriguez *et al.*, 1999; Prystupa *et al.*, 2003). One possible reason for this difference in approach is that analysing the fate of floret primordia is far more difficult and time-consuming than determining tiller numbers at different times. However, it may be critical in crops such as wheat in which grains per m² are frequently related to the number of grains per spike, rather than to the number of spikes per m² (De Vita *et al.*, 2007; Elhani *et al.*, 2007). This is probably due to the fact that wheat has an indeterminate number of florets per spikelet, allowing the adjustment of the number of offspring to the environmental conditions through large changes in survival/death of floret primordia, determining the number of fertile florets that may produce a grain (e.g. Kirby, 1988).

The rather difficult detailed determinations needed for the analyses of floret generation/degeneration processes requires a focus on developmental features of only a few florets, presumably representing the variation that is seen in the canopy. Fortunately, it has been shown that there is a reasonably good integration from the fate of individual florets to the number of florets and grains of the crop (see González *et al.*, 2005a) through either genotypic differences associated with Rht (Miralles *et al.*, 1998) or Ppd alleles (González *et al.*, 2005b) or the manipulation of the duration of (González *et al.*, 2003; Serrago *et al.*, 2008), or radiation intensity during (González *et al.*, 2005a), the stem elongation phase, when most of these floret developmental processes take place (Kirby, 1988).

To the best of our knowledge, there are almost no studies published relating floret primordia generation and survival to N availability in wheat [the unique exception being the descriptive study by Sibony and Pinthus (1988) in bread wheat], and no studies seem to be available for floret developmental processes, determining differences in grains per spike, in durum wheat. It is hypothesized that environmental factors that increase grain number would do so through reducing the mortality of floret primordia, based on the fact that producing floret primordia during the floret initiation phase would have a much lower energetic cost in

relation to that of floret survival in the degeneration phase (due to the difference in size and number of grains growing in these two cases).

To test the hypothesis, the dynamics of floret primordia development of durum wheat in response to N under contrasting water regimes were analysed.

Materials and methods

General conditions

Two experiments were carried out outdoors in microcrops within large rectangular containers (1 m height and 1×1 m² surface; Fig. 1) in the premises of the School of Agronomy, University of

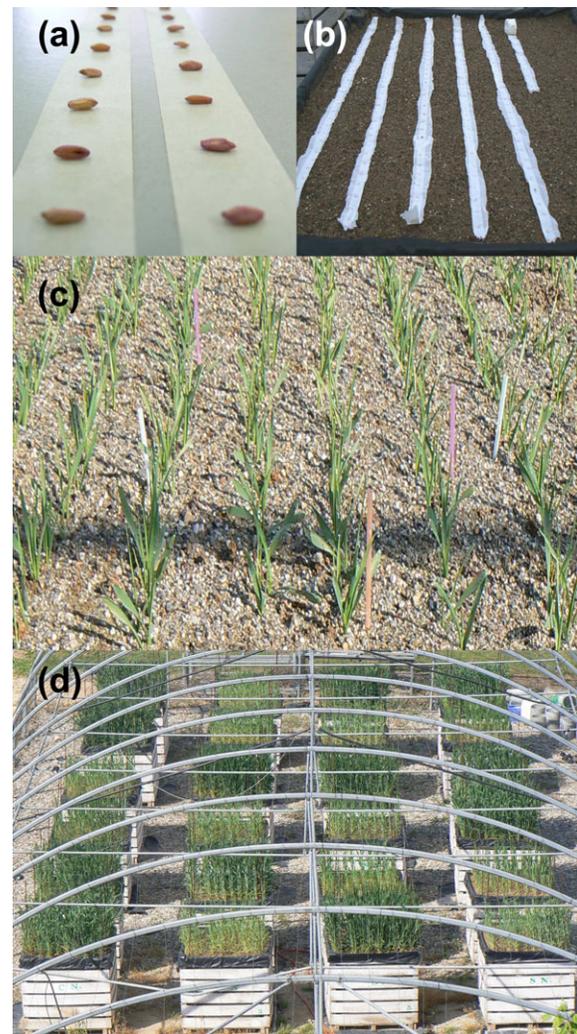


Fig. 1. Images illustrating different aspects of the preparation and setting up of the experiments. Seeds were manually placed at precise regular intervals, to maximize uniformity of the stand, on masking tape (a), then these 1 m linear strips were covered with tissue paper and placed in the rows of each experimental unit (b). The consequence of the delicate handling for sowing was the achievement of experimental units with almost perfectly uniform microcrops (c). (d) The general appearance of the experiment with all the experimental units covered with an anti-bird mesh.

Lleida, Spain (41°37' 50" N, 0°35' 27" E, 180 m). Experiment 1 was conducted in 2006–2007, and experiment 2 in 2007–2008. In order to ensure a low availability of N, the containers were filled with a sand:soil mix (3:1 v/v). In each of the two studies, N-nitrate in the soil mixture at the beginning of the experiment was low. In the whole container mineral N availability was equivalent to 70 kg N ha⁻¹ in experiment 1 and 30 kg N ha⁻¹ in experiment 2.

Microcrops were sown within the optimal sowing period for cereals in the region, on 24 November 2006 (experiment 1) and on 14 November 2007 (experiment 2). Within each container crops were sown in rows, 10 cm apart. To ensure maximum uniformity within each microcrop, seeds were placed manually on 1 m linear strips of masking tape (Fig. 1a) and then covered with tissue paper. These strips were placed in the rows and covered with the soil mixture (Fig. 1b).

The density was 500 plants m⁻² (experiment 1) and 300 plants m⁻² (experiment 2). P fertilizer (triple superphosphate, 20 kg P ha⁻¹) was uniformly mixed within the upper 20 cm of the soil mixture before sowing in each experiment. Weeds were removed by hand throughout the growing season. Diseases and insects were prevented by spraying recommended fungicides and insecticides at the doses suggested by their manufacturers.

Treatments and experimental design

Treatments consisted of a durum wheat (cv. Claudio, chosen to be representative of those with good performance under field conditions in previous studies; e.g. Cossani *et al.*, 2007) subjected to the factorial combination of two levels of N and two levels of water availability. The N treatments were a control without fertilization (N₀) and a fertilized treatment which received 100 kg N ha⁻¹ (experiment 1; N₁₀₀) or 250 kg N ha⁻¹ (experiment 2; N₂₅₀). N fertilizer (urea) was applied together with the earliest irrigations, splitting the dose to avoid leaching, in two (experiment 1) or three applications (experiment 2) at different developmental stages using the decimal code (DC) from Zadoks *et al.* (1974). These stages were the initiation of tillering (DC 2.1) and the initiation of stem elongation (DC 3.1) in both experiments, and in experiment 2 there was a third application between them (DC 2.3). The two water treatments consisted of rainfed (watered only once at sowing to ensure germination and emergence, and when N was applied) and irrigated conditions (maintained near field capacity by periodic irrigations throughout the growing cycle). Due to the excessive rainfall during pre-flowering in experiment 1, especially from jointing to flowering (Fig. 2), the water treatment was not significant and was thus only analysed in experiment 2 (i.e. in experiment 1 only N was an environmental factor). Treatments were arranged, within each experiment, in a complete randomized design with three replicates. Each microcrop was an experimental unit.

Measurements and analyses

Once or twice a week, one plant per experimental unit was randomly harvested and its main shoot was dissected to determine the timing first of the double flake formation and later that of terminal spikelet initiation (Kirby and Appleyard, 1984). The timings of the beginning of stem elongation (DC 3.1), flowering (anthesis; DC 6.5), and physiological maturity (DC 9.5) were recorded when 50% of plants in a microcrop reached that stage.

From terminal spikelet to flowering, one plant per experimental unit was selected at random and harvested twice or three times a week. The main spike was dissected to count the total number of floret primordia under a binocular microscope (Leica MZ 6, Leica Microscopy System Ltd, Heerbrugg, Switzerland). In addition, at each sampling, the floral score of each floret within particular spikelets was determined following the scale of Waddington *et al.* (1983), mostly based on pistil development from stage W3.5 (stamen primordia present) to stage W10 (styles curved and stigmatic branches spread wide, pollen grains on well-developed

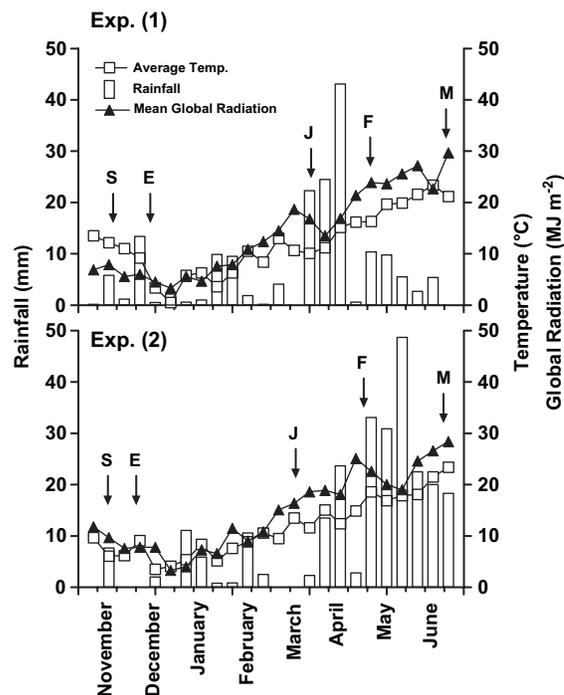


Fig. 2. Accumulated rainfall, mean temperature, and mean daily global radiation for 10 d periods during the 2006–2007 (experiment 1, top panel) and 2007–2008 (experiment 2, bottom panel) growing seasons at the experimental site (Lleida, Spain). Arrows indicate the average dates of sowing (S), seedling emergence (E), jointing (J), flowering (F), and maturity (M) for wheat.

stigmatic hairs). Floret primordia were considered as fertile florets when they were at W10 (Fig. 3) or immediately before that stage (when the stigmatic branches were curved with green anthers). The spikelets analysed were those in the basal (fourth spikelet from the base of the spike), central (middle spikelet position of the spike), and apical (fourth spikelet from the top of the spike) positions of the spike. Effects of N and water treatments on the number of spikelets per spike were insignificant. Naming of florets within the spikelets followed the same system described by González *et al.* (2003); that is, from F1 to F_n regarding their position with respect to the rachis, F1 being the floret closest to the rachis. The rate of floret development ([°C dJ⁻¹) was calculated as the reciprocal of the thermal time required to progress from W3.5 to W10. Thermal time was calculated assuming a base temperature of 0 °C.

Four plants were tagged in each container and the number of tillers per main culm was counted twice a week in experiment 2.

At physiological maturity, aboveground biomass was harvested in 2 m (4×0.5 m in the inner rows) in each experimental unit. Aboveground biomass was separated into culms and leaves, spikes and grains, and oven-dried at 65 °C during 48 h and then weighed. Grain yield and its main components were determined.

To determine the effect of treatments on the different variables, the data were subjected to analysis of variance and the relationships between variables were determined by regression analysis (SAS statistics program, 2001).

Results

Yield and yield components

As expected, increasing N availability resulted in higher aboveground biomass and grain yield at maturity in both

experiments (Table 1). Increasing water availability in experiment 2 also resulted in higher biomass and grain yield (Table 1). Although the effect of N tended to be higher in irrigated than in rainfed crops, the magnitude of the interaction was negligible compared with that of the main

factors for yield and biomass, or even non-significant for yield components (Table 1). Therefore, the results were focused on the effect of the main factors.

Yield was better related to the number of grains m^{-2} ($R^2=0.95$, $P <0.05$) than to individual grain weight

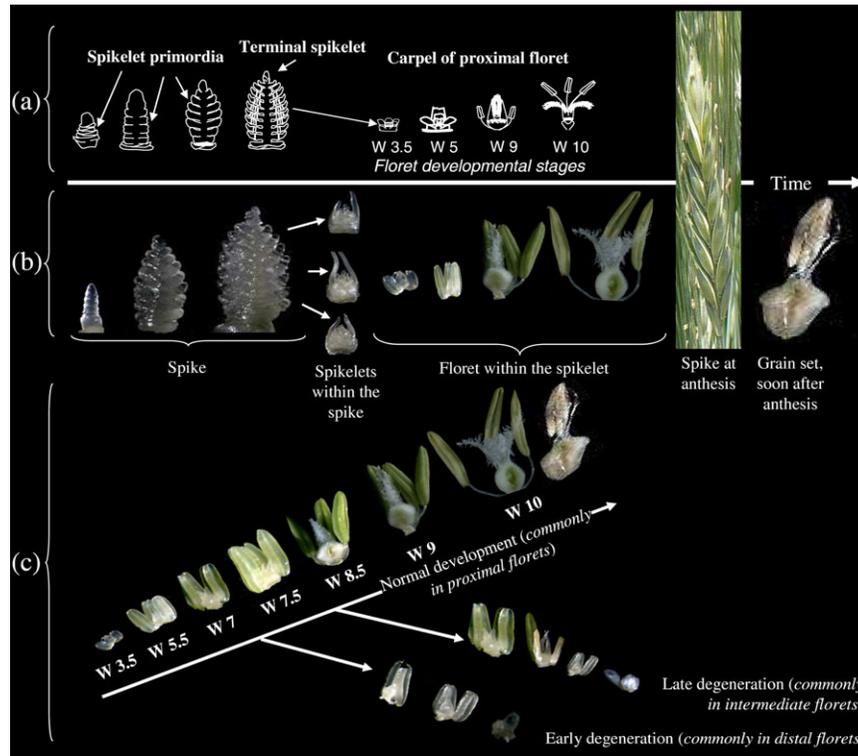


Fig. 3. Illustration of wheat floral development as shown schematically (a) and in images taken during the experiments (b) from early spikelet primordia differentiation through terminal spikelet to anthesis, with details of selected floret developmental stages. In both panels (a and b) first the development of the spikelets within the spike is shown up to terminal spikelet initiation, and from then the development of floret primordia (that successfully reach the stage of fertile florets at anthesis) within spikelets is shown. In b, there is also an illustration of the different degree of development of spikelets selected to analyse the developmental progress of its floret primordia. The bottom panel (c) illustrates the floral development dynamics with time (upward-facing arrow) for florets that develop normally towards achieving the stage of fertile floret at anthesis and setting grains afterwards. The downward-facing arrows show the floral degeneration process either early or late during development, as observed microscopically during the experiments. The pictures and drawings are not to scale; as a reference, the width of a floret in W 3.5 is ~0.10 mm, in W5 0.15 mm, in W7.5 0.30 mm, and in W10 1.60 mm.

Table 1. Yield, biomass, and main yield components in the experiments carried out in Lleida (NE Spain) during 2006–2007 (experiment 1) and during 2007–2008 under irrigated and rainfed conditions (experiment 2).

For experiment 2 the mean square (MS) for the effects of nitrogen (N), water (W), and their interaction (N×W) are also shown.

		Yield ($g\ m^{-2}$)	Biomass ($g\ m^{-2}$)	Grain number ($10^{-3}\ m^{-2}$)	Grain weight ($mg\ grain^{-1}$)	No. of grains ($spike^{-1}$)	No. of spikes (m^{-2})	
Experiment 1	N ₀	272 b	640 b	7.59 b	36.0 a	16.9 b	453 b	
	N ₁₀₀	512 a	1172 a	14.12 a	36.2 a	28.0 a	504 a	
Experiment 2	Irrigated	N ₀	283 b	637 b	6.82 b	41.9 a	22.4 b	304 b
		N ₂₅₀	727 a	1528 a	16.13 a	45.0 a	35.8 a	453 a
	Rainfed	N ₀	235 b	664 b	5.91 b	39.7 a	18.7 b	315 b
		N ₂₅₀	518 a	1096 a	12.77 a	40.6 a	31.0 a	413 a
		MS _(N)	396 758***	1 314 481***	196 291***	12.5 NS	491***	45 757***
	MS _(W)	49 676***	122 964***	13 675*	32.4 NS	54.7 NS	638 NS	
	MS _(N×W)	19 282*	157 493*	4499 NS	3.73 NS	1.17 NS	1875 NS	

Different letters within a column stand for significant difference between Nitrogen regimes within each experiment and water regime.

Bold figures indicate that irrigation significantly affected the variable within each N regime.

*, **, ***, and NS stand for the level of significance of the MS values (0.05, 0.01, 0.001, and non-significant, respectively).

($R^2=0.01$, non-significant). In turn, the number of grains per m^2 was better related to the number of grains per spike ($R^2=0.81$, $P < 0.05$) than to the number of spikes per unit land area (Table 1).

Taken together, most of the large effects of N and water on wheat yield were due to the effects on the number of grains per spike. Then, to ascertain the origin of the yield responses to treatments, the focus turned to the analysis of the fate of floret primordia within the spikes, which ultimately determines the number of fertile florets (most of which are later grains) per spike.

Dynamics of living florets

The number of living florets (i.e. initiated floret primordia that continued developing normally towards more advanced developmental stages at each sampling) was always increased in response to increased availability of resources (Fig. 4), although the magnitude of the effect, as well as the specific patterns, varied slightly between the experiments.

In experiment 1, higher N availability resulted in an increased number of fertile florets in apical and basal spikelets, while it was mostly unaffected in central spikelets (Fig. 4, left panels); in experiment 2, the number of fertile florets increased more markedly, and more consistently, in

the central than in the basal/apical spikelets in which fertile florets were only slightly increased when experimental units were irrigated (Fig. 4, central and right panels). Overall, there was not a single and uniform shift in the pattern of floret initiation in response to N, with a trend to increase the number of florets developing normally with higher N availability in experiment 1, but not in experiment 2 (Fig. 4). What did seem to be consistent across the three background environmental conditions was that the improved number of fertile florets at the end of the floret-developing period in response to N was related more to the survival of florets that were initiated than to the maximum number of floret primordia initiated (Fig. 4).

Fate of floret primordia

The details of the developmental dynamics of particular floret primordia that produced the responses to N shown in the number of living (and, at the end, fertile) florets, evidenced that in experiment 1 increased N availability resulted in a slight trend towards an increased rate of floret development in almost all spikelets and florets (Fig. 5a). The two most proximal florets reached the fertile floret stage at flowering in all spikelets irrespective of the N treatment, but in F3 from the rachis the advanced

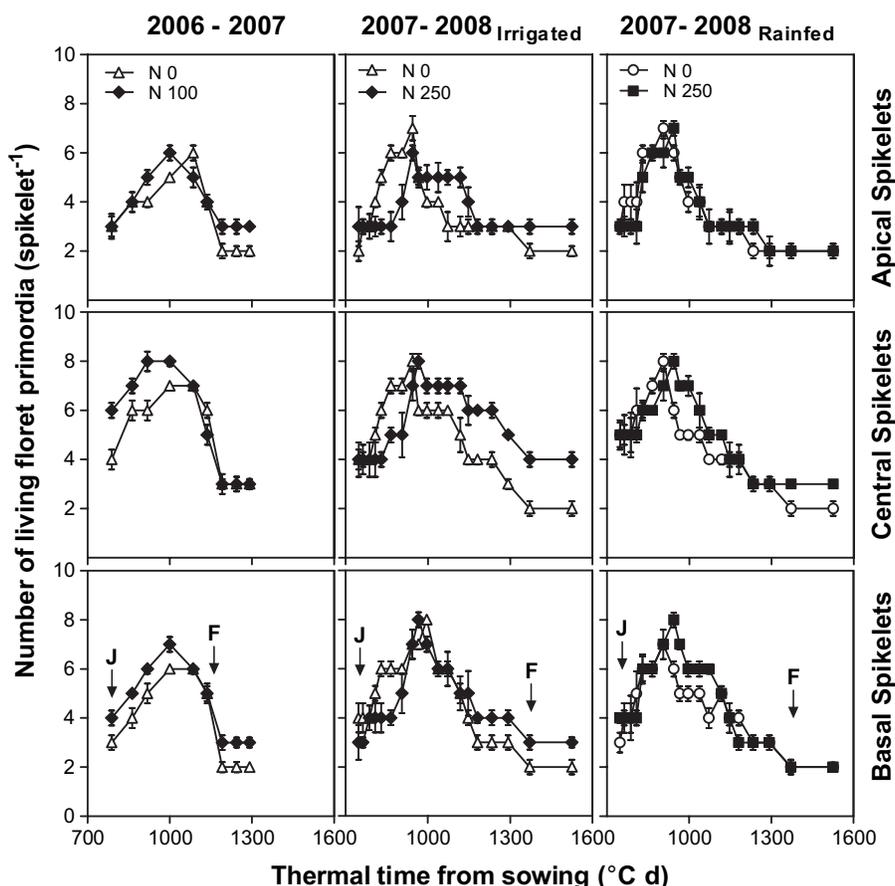


Fig. 4. Dynamics of the number of living floret primordia from jointing to flowering under high and low N availabilities (filled and open symbols, respectively) for basal, central, and apical spikelets of the main shoot spikes of durum wheat in experiments carried out in Lleida (NE Spain) during 2006–2007 (experiment 1) and during 2007–2008 under irrigated and rainfed conditions (experiment 2).

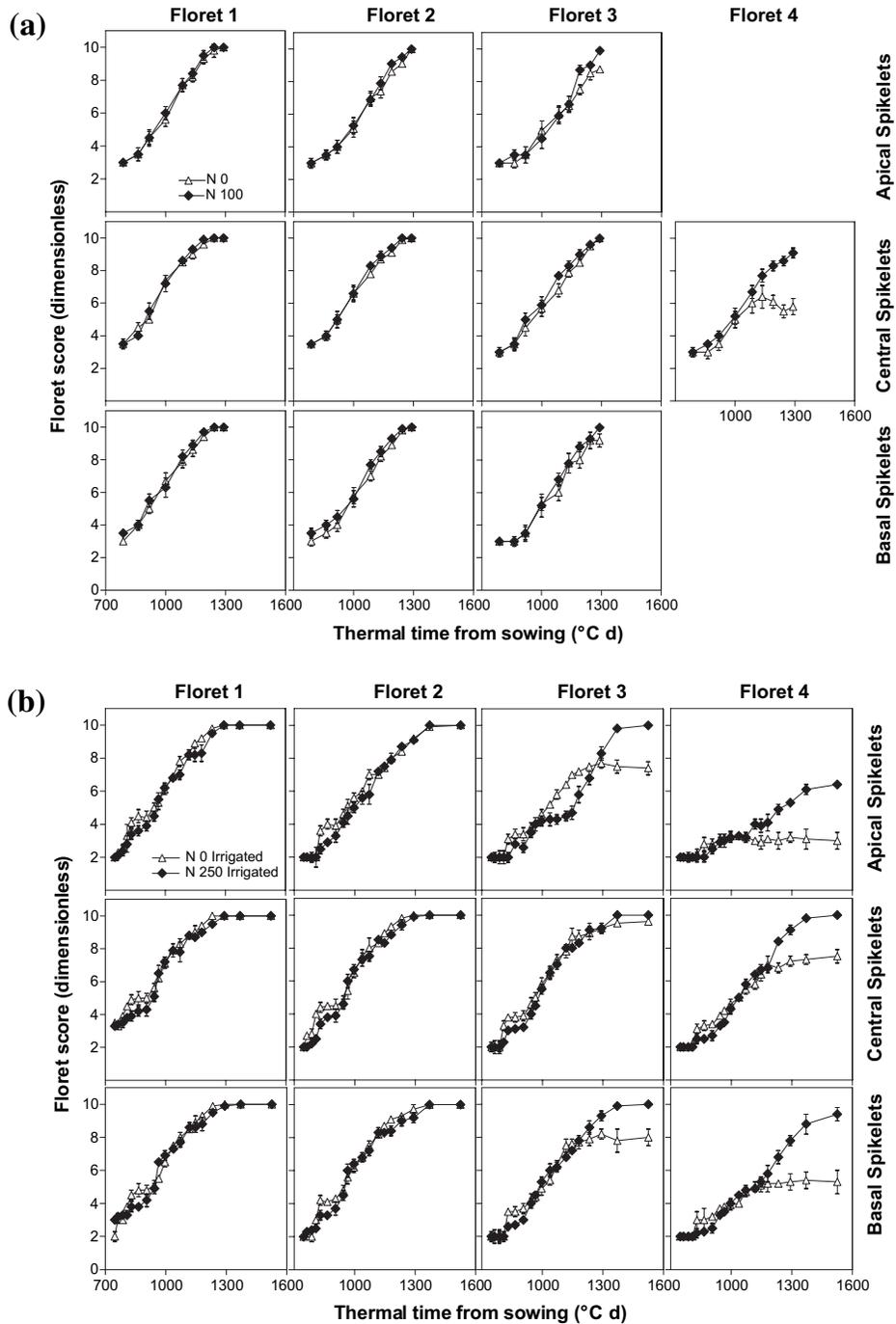


Fig. 5. Effect of nitrogen availability on floret development of durum wheat during the stem elongation phase after jointing for the 3–4 florets most proximal to the rachis florets in each of the three spikelet categories considered for experiments carried out in Lleida (NE Spain) during 2006–2007 (a) and during 2007–2008 under irrigated (b) and rainfed conditions (c). Floret development was assessed through frequent determination of floret stages following the scores given by the scale of Waddington *et al.* (1983).

development produced by N ended up in setting a third fertile floret in basal and apical spikelets, while under low N these third florets did not reach the W10 stage (Fig. 5a); and in the central spikelets the third floret reached the fertile stage under both N conditions. Even though accelerating the developmental rates did not increase the number of fertile florets in the central spikelets of experiment 1, this lack of effect was only relevant in qualitative terms (existence or not of a fertile floret) while in quantitative

terms N affected floret development similarly in all spikelets as can be illustrated by the developmental progress of the fourth floret in the central spikelets: although florets in this position did not reach the stage of a fertile floret in any of the N treatments, the difference produced by N availability in their development was quite noticeable (Fig. 5a). The effect in all other floret primordia (those in positions more distal than F4) also showed that the stage of development reached was always more advanced if more N was available

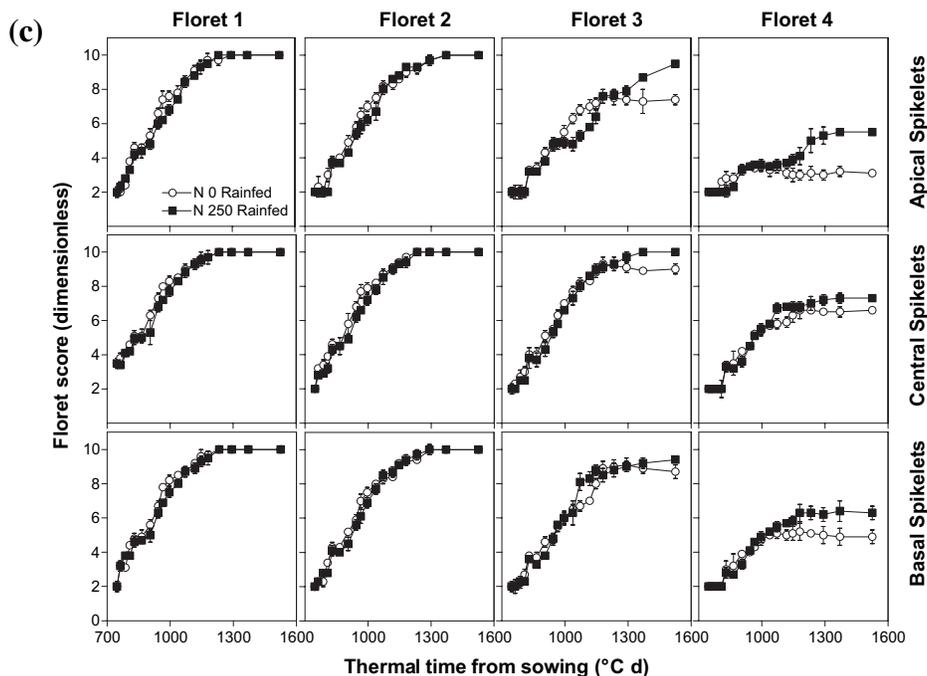


Fig. 5. Continued

(data not shown), although none of them reached stages of development close to fertile florets. Thus, although when considering the final outcome in terms of number of fertile florets the effect of N was concentrated on basal and apical spikelets, when considering developmental dynamics of all floret primordia (including those not reaching the fertile floret stage) the effect of N was similar in all spikelets.

In experiment 2 it seemed that the effect of N accelerating developmental rates of floret primordia was only seen after a slight delay in the initiation of fast development (Fig. 5b, c). In other words, for some reason the initiation of the fast rate of development of the different floret primordia was anticipated in the N-stressed conditions, but, from the onset of the fast rate of floret development onwards, the high-N treatment produced a faster rate of floret development than the low-N treatment, so that even after starting development later the florets reached the fertile stage together with, or earlier than, those under N stress (Fig. 5b, c). Under unrestricted water availability, florets 1 and 2 of all spikelets reached the stage of fertile florets in all spikelets analysed, but then F3 of apical and basal spikelets, as well as F4 of central spikelets, reached the fertile floret stage only if N was high (Fig. 5b), and, even when floret 4 of the apical and basal spikelets did not reach the fertile floret stage, it was clear that N accelerated developmental processes of these florets. When experimental units were rainfed, the two most proximal florets (F1 and F2) also reached W10 in all spikelets (Fig. 5c), while the third floret reached the stage of a fertile floret only in the central spikelet in high N (Fig. 5c), explaining the difference in number of fertile florets in these spikelets (Fig. 4, right panels). Once again, under both water regimes, analysing the fate of florets that were more distal and which did not

reach the stage of a fertile floret, the effect of N availability in improving their developmental rates is still clear, as shown for floret 4 in apical and basal spikelets under irrigation (Fig. 5b) and in all spikelets under rainfed conditions (Fig. 5c), as well as consistently happened with florets in more distal positions under both water regimes (not shown).

Discussion

In agreement with most agronomic literature, grain yield was higher when N and water availability increased. Despite the fact that the present experimental conditions were not just field plots, the observed yields were quite in line with what could be expected from realistic field crops in the region (e.g. Cossani *et al.*, 2007). Also in line with what is frequently reported in the literature, yield responsiveness to N and water was the consequence of an increased number of grains per unit land area, as commonly happens in cereals elsewhere (e.g. Fischer, 1993; Prystupa *et al.*, 2004) including in Mediterranean environments (Cossani *et al.*, 2009). This is because in cereals the average weight of the grains is far more stable than the number of grains per m² (e.g. Egli, 1998; Peltonen-Sainio *et al.*, 2007).

Furthermore, the increase in grain number was mainly related to increases in grains per spike, not only under high planting densities such as were used here in the first experiment, but also under the relatively lower density used in the second experiment. Although plant density was high, and particularly in experiment 1 was higher than what is usual agronomically, it is believed that the main outcomes may be extrapolated as durum wheat has a relatively

moderate tillering capacity (De Vita *et al.*, 2007; Elhani *et al.*, 2007) which could explain why the determination of the number of grains per spike is critical in determining the magnitude of the crop responsiveness to availability of resources. This sets up the context in which understanding the generation of grain number per spike in response to N and water is closely equivalent to understanding yield physiology.

The number of grains per spike is mainly the consequence of the dynamics of floret initiation and degeneration to produce fertile florets at flowering (Kirby, 1988). In this study it was shown that increasing N availability diminished the amount of floret primordia degenerating during the late part of the stem elongation phase. The fact that the number of floret primordia initiated was less relevant than the rate of floret degeneration for establishing the number of grains in response to N is consistent with what has been found to be the mechanism by which either the introgression of dwarfing genes (Miralles and Slafer, 1995) or the exposure to short photoperiod (González *et al.*, 2005a) increased grain number per spike. In all these cases, as well as when modern and old cultivars are compared (e.g. Slafer and Andrade, 1993), the final number of grains per spike (and per m²) seems far more related to floret survival processes, as, in most cases, as well as in the present study on responsiveness to N, the number of floret primordia initiated was similar among treatments. This general pattern may reflect the fact that the energy cost to initiate floret primordia must be markedly lower than that required to allow normal development of the florets determining the rate of floret survival, or the fact that during floret initiation the demand for assimilation by competing organs (markedly so for stems) is much less than during the floret survival period. In fact, there should be an evolutionary advantage in plants producing a very large number of primordia and then, at the time when the number of fertile florets is determined, to allow the survival of those that could be safely filled (and produce offspring). This speculation fits not only with the pattern described here, but also with the proposal of Sadras (2007) regarding the evolutionary aspects of crops having a remarkable plasticity of seed number in relation to availability of resources, estimated through parent growth rate, during critical stages. In wheat the most critical phase for yield determination is that immediately preceding flowering (e.g. Fischer, 1985; Savin and Slafer, 1991; Miralles and Slafer, 2007).

It is concluded that the well known effects of N fertilization in improving cereal yields, when grown in N-deficient conditions, related to the growth of the juvenile spikes (Fisher, 1993; Prystupa *et al.*, 2004) operates by allowing a more accelerated rate of floret development, which is the cause of a higher rate of survival of the rather large number of floret primordia that are normally initiated in all spikelets of wheat. This confirms that floret survival is a major determinant of grain number in wheat and that the process seems to be mediated by resource availability, as previously suggested by Gonzalez *et al.* (2005a) from studies based on more manipulative types of treatments imposed on hexaploid wheat.

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