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Tillering and floret development dynamics in wheat cultivars of contrasting spike fertility plasticity

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ABSTRACT

Context: Given the significant impact of grain number (GN) on wheat grain yield, it is critical to gain a deeper understanding of the physiological bases of GN determination. The formation of both spike number (SN) and grains per spike (GS) coincides temporally and undergoes a parallel process characterized by the extensive generation of structures (such as tillers or floret primordia), succeeded by substantial degeneration, ultimately leading to a limited number of viable spike-bearing tillers and fertile florets at anthesis. Survival highly depends on resource availability and the existing genetic variability may result in varied SN and GS response to inputs or environmental conditions.

Objectives: We examined the dynamics of tillering and floret primordia initiation and death during late reproductive phase aiming to elucidate the mechanisms underlying the differences in SN and GS plasticity in wheat. **Methods:** The number of living tillers and floret primordia developmental stages were recorded periodically on two contrasting genotypes (Ascott and Sy Moisson) regarding their GN components plasticity in response to two levels of shading, two levels of thinning and an unmanipulated control, over two locations (France and Spain). Treatments started at the onset of stem elongation. Shading finished at anthesis.

Results: Survival of tillers and floret primordia rather than their generation was key for the responses to resource availability. Ascott exhibited higher plasticity which was attributed to enhanced tiller and floret primordia survival response, suggesting its superior resource utilization and adaptability to changing conditions. The higher response of florets was observed particularly in the basal spikelets, a portion of the spike that is typically less fertile. Our results emphasize the importance of the final stages of the late reproductive phase to the determination of GN (as during this period the degeneration process occurs) and also corroborates with the evolutionary idea that overproducing primary reproductive structures does not suppose any disadvantage.

Conclusions: Plasticity of GN determinants were mainly related to the responsiveness of survival of initiated structures for both SN and GS. Tiller and floret primordia survival strongly responded to the treatments and the genotypic differences in the spike fertility were mainly related to responses in the basal portion of the spike. **Implications or significance:** Overall, this study contributes with insights into the physiological bases of GN determination, offering implications for wheat breeding strategies aimed at improving grain yield and adaptability to diverse environments.

1. Introduction

In wheat (as well as in many other crops) grain growth is most frequently sink-limited during the effective period of grain filling (e.g., Borrill et al., 2015; Serrago et al., 2013; Slafer et al., 2023). Therefore, grain yield (GY) is mostly related to grain number per m² (GN,

Peltonen-Sainio et al., 2007; Sadras, 2007; Slafer et al., 2014) which is far more plastic than the average weight of the grains (Lenoir et al., 2023; Sadras and Slafer, 2012). Gains in GY would be facilitated through a better understanding of the physiological bases of GN determination (Slafer et al., 2022). GN can be dissected in grains per spike (GS) and spike number per m² (SN) whose formation overlap in time (Hay and

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Kirby, 1991) and strongly depend on resource availability (Slafer et al., 2021).

There is genetic variability among elite materials (frequently high-yielding modern cultivars) that may differ in the relative relevance of particular yield components (e.g., Beral et al., 2022; Bustos et al., 2013; Kim et al., 2024) which can be crossed to improve GY. These genotypes may vary their SN and GS plasticity; i.e. their response to the inputs or environmental conditions. In the companion paper (Bicego et al., 2024), we experimentally proved the differential plasticity of GS and SN of two modern cultivars (Ascott and Sy Moisson that had been claimed from multiyear-multilocation comparisons of cultivars performance). We evidenced through manipulating the availability of resources during stem elongation that (i) both components of GN were more plastic in Ascott than in Sy Moisson, and (ii) the different plasticity of GS was reflected in the fertility of the spikes at anthesis (i.e. to the number of fertile florets per spike; FF), through a linear relationship between FF and spike dry weight at anthesis (SDWa). Although SN and FF may be measured at anthesis, they are determined through processes that started much earlier (Kirby, 1988). Studying the dynamics of generation of these components and their plasticity would contribute to understand better the physiological bases of GN determination.

SN is the outcome of a process that starts after the onset of tillering, and for each individual plant within the canopy it is conditioned by plant density. The first tiller usually appears as the second to fourth leaf emerges (Alzueta et al., 2012) and from then on tillering goes in coordination with leaf appearance (Slafer et al., 2021), if there are no resource limitations for the growth of younger tillers (Power and Alessi, 1978). That is why tillering does usually end around the onset of stem elongation (when a large amount of resources are diverted for stem growth; Xie et al., 2016). And soon after the end of tillering under agronomic plant density (which maximizes yield for the particular condition) the tiller mortality phase initiates (Hucl and Baker, 1989). From the onset of stem elongation to anthesis a significant proportion of the appeared tillers die, being tiller survival a major determinant of SN (Dreccer et al., 2013).

Similarly, and with significant overlapping in time, GS formation goes through a process in which (i) firstly spikelets are initiated from floral initiation (some time before double ridges; Baker and Gallagher, 1983) to terminal spikelet, commonly coinciding with the onset of stem elongation (Kirby et al., 1994), and then (ii) a huge number of floret primordia are generated within the spikelets followed by a very large degeneration of many of them, where only a few primordia continue developing normally to produce fertile florets at anthesis (Ghiglione et al., 2008; Prieto et al., 2018). The differentiation is sequential within each spikelet, but the floret primordia of the central spikelets are initiated before the terminal spikelet is initiated, then the starting of the floret initiation process is overlapped with the final part of spikelet initiation. While floret initiation seems to be genetically driven and not strongly affected by resources (i.e. the maximum number of floret primordia initiated seems largely unresponsive to resource availability; Ferrante et al., 2010), the survival of floret primordia seems related to the allocation of resources to the growing juvenile spike (González et al., 2005); as expected theoretically from an evolutionary point of view (Sadras and Slafer, 2012). Indeed, the onset of floret mortality seems related to the initiation of active growth of the juvenile spikes (González et al., 2011; Zhang et al., 2021), rather than to the stage of development of the most advanced florets (Ferrante et al., 2013b), as had been proposed earlier (Bancal, 2009). Thus, it is not surprising that a strong relationship has been frequently reported between the number of grains or fertile florets and the SDWa, reflecting the outcome of floret survival in line with spike growth (Slafer et al., 2023).

In this paper we reported the genotypic differences in dynamics of both tillering and spike growth-development during the stem elongation phase, the processes that precede, and mechanistically determine, SN and GS, in response to availability of resources for the two cultivars selected to differ in plasticity of GN components.

2. Materials and methods

Details of the experiments, treatments and general determinations are available in the companion paper (Bicego et al., 2024). To recap the most critical aspects, two sister field experiments were conducted in Bell-Lloc d'Urgell (Lleida, NE Spain, sown on 24 November 2020 with a final density of 250 plants m^{-2} , after manually thinned the plots at seedling emergence to maximize uniformity) and Clermont-Ferrand (France, sown on 06 November 2020 with 190 plants m^{-2}). Both experiments were irrigated and fertilized as required to avoid water-nutrient stress and pests were controlled. In both experiments treatments consisted of two modern commercial cultivars with similar average yields and phenology, that in multi-environmental experiments exhibited contrasting plasticity of GS to changes in SN (Ascott more plastic than Sy Moisson), were exposed to 5 levels of resource availability at the onset of stem elongation: shading 50 % (Sh50), shading 25 % (Sh25), unmanipulated control (Ctrl), thinning 50 % (Th50), and thinning 75 % (Th75). Broadly speaking, these treatments would correspond to 0.5-, 0.75-, 1-, 2-, and 4-fold the available resources in the control, respectively (see Fig. 2 in the companion paper; Bicego et al., 2024). Please note that this is a broad approach to changes in availability of resources (required to allow us to estimate plasticity as described in the companion paper), particularly when considering thinning (that would have increased radiation interception per plant proportionally less than the increase in land area per plant; see Discussion in Bicego et al., 2024). When analyzing the response of florets within spikes to treatments, we considered the relative resource per spike normalizing the relative resources per plant (0.5, 0.75, 1, 2, and 4) by the number of spikes per plant in each treatment.

Shading treatments were removed at anthesis (i.e., shading treatments were imposed from the onset of stem elongation to anthesis), while the thinning treatment, consisting on the removal of every other row of the plot (Th50) and additionally removing every other plant within the remaining rows (Th75), naturally remained in that condition until maturity. In Lleida, to impose the shading treatment, wood structures (1 m height, 1.2 m width and 4 m long) were built in each designated plot and covered with the selected cloth. In Clermont-Ferrand, the treatment was applied in a similar manner, but covering 60 % of the plot, within which samples were further collected. All the treatments were consistently applied with no variations in the implementation of the shading or thinning treatments for the two cultivars and across both locations, ensuring uniformity in the experimental conditions. Treatments were arranged in a completely randomized block design with three replicates in both locations. Plots in Lleida consisted of 6 rows, 0.2 m apart and 4 m long, while in Clermont-Ferrand they were 8 rows, 0.17 m apart and 5 m long. Daily incident solar radiation and average temperature from the onset of stem elongation to anthesis are shown for both Clermont-Ferrand and Lleida (Fig. 1). The time course of processes analyzed were based on thermal time, simply adding daily the average temperature recorded at each experiment (assuming a base temperature of 0°C).

SN and GS were determined at physiological maturity and FF at anthesis from crop-level samples taken in each experimental unit. The number of spikes was determined from counting them in the sample (1-m long of a central row in Lleida and 50 consecutive spikes from the center row in Clermont-Ferrand). We separated the main shoot and tiller spikes of the sample. The samples were oven dried for 48 h at 65°C, then threshed and the number of grains per spike was calculated.

In addition, we also determined in Lleida the dynamics of both (i) tillering and tiller survival, (ii) floret initiation and mortality, and (iii) dry weight of the growing juvenile spikes. In Clermont-Ferrand, the maximum number of floret primordia initiated and the number of fertile florets were determined and the floret survival rate calculated at the same contrasting spikelets selected in the experiment carried out in Lleida.

The dynamics of tillering and tiller mortality were derived from

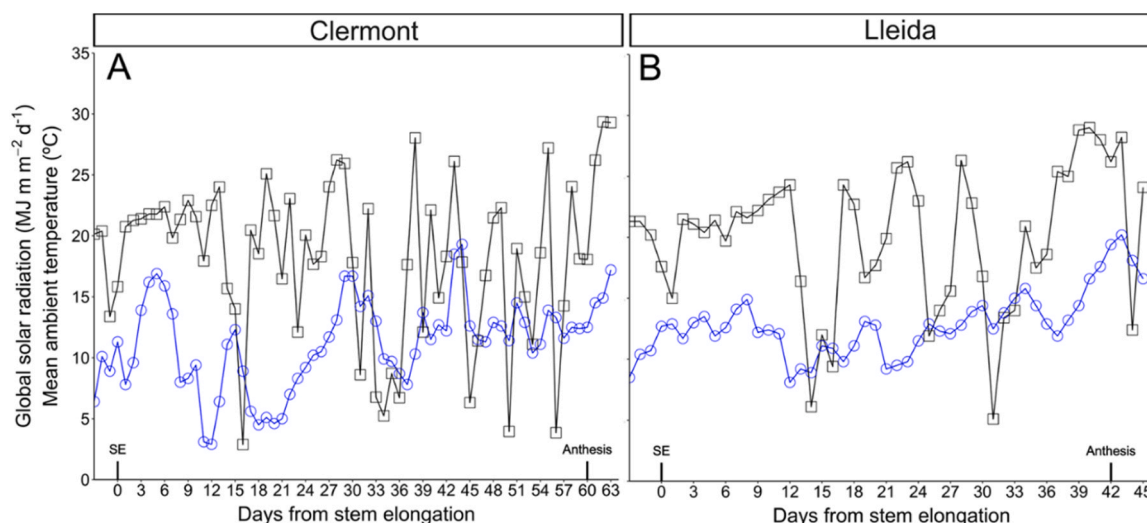


Fig. 1. Daily mean temperature (blue circles) and global radiation (black squares) from the onset of stem elongation (SE) in Clermont-Ferrand (A) and Lleida (B). The period from the onset of stem elongation to anthesis was longer in Clermont-Ferrand than in Lleida (because the weather was cooler in Clermont-Ferrand). Cultivars showed very similar phenology within each location: in Clermont-Ferrand, for both cultivars, the dates of the onset of stem elongation and anthesis were 26 March and 25 May respectively. In Lleida, for both cultivars stem elongation occurred on 26 March and anthesis on 6 May for Sy Moisson and 8 May for Ascott.

measurements of the number of shoots (main shoot and emerged tillers) at weekly intervals from the onset of tillering to anthesis and then at maturity. The determination was done non-destructively firstly (from the onset of tillering to the onset of stem elongation), and from destructive samples from then on. In the first period, we counted periodically the number of shoots in a randomly chosen single portion of 0.25 m of a central row in each plot (c. 13 plants). From the onset of stem elongation to anthesis, we took samples of 0.25 m of a central row of each plot from which we (i) counted the number of plants and shoots and then (ii) separated the spikes from the main shoots, through dissecting the shoots to extract the juvenile spikes, and determined the dry matter of the shoots in general and of the spikes in particular, after oven-drying the samples for at least 48 h at 65 °C.

In Lleida, the dynamics of floret development and in Clermont-Ferrand the maximum number of floret primordia and the number of fertile florets were determined. For the dynamics of floret development, we took several samples from terminal spikelet to anthesis. One representative plant per experimental unit (three plants per treatment) was sampled randomly twice a week and taken to the laboratory for further dissection under a microscope (Leica MZ 7.5, Leica Microscopy System Ltd., Heerbrugg, Switzerland). The characterization of floret primordia development was performed in three specific spikelet positions of the spike: basal (4th spikelet from the base), central (middle of the spike), and apical (4th spikelet from the terminal spikelet down). For each floret primordium (of each position) a developmental score was recorded based on the scale developed by Waddington et al. (1983) that mainly considers the changes in pistil morphology to establish the floret developmental stage. Florets were numbered, within each spikelet, from 1 (the closest to the rachis) to n (the most distal position from the rachis). The number of living florets for each sampling represents the number of florets that were actively developing at that time. At anthesis, from a sample of 1 m of a central row (for details see Bicego et al., 2024) the number of fertile florets was counted in all spikelets to determine the number and distribution of fertile florets per spike. In Clermont-Ferrand, the number of floret primordia in basal, central and apical spikelets was determined at booting stage (DC 45), when 5 spikes were randomly sampled from the central rows of each experimental unit and dissected under microscope to determine the number of floret primordia initiated at these spikelets. This was considered the maximum number of floret primordia initiated, as it is when floret mortality becomes more evident (Kirby, 1998). At anthesis, the same process was repeated to determine

the number of fertile florets in those basal, central and apical spikelets.

The response of each particular trait to the treatments was calculated as relative values respect to the control within each genotype: for each cultivar, the control plot was fixed as the unity and the response to a given particular resource availability treatment was expressed as the ratio of its absolute value on that of the control.

3. Results

3.1. Dynamics of tillering and tiller death

It was shown in the companion paper (Bicego et al., 2024) that the thinning treatments increased significantly the number of spikes. The treatments were applied at the onset of stem elongation, which under field conditions of commercial crops (i.e., sown at optimum dates and plant densities) roughly coincides with the end of tillering and is followed soon by the senescence of late formed tillers. This was true for all treatments but for Th75 (having c. 4-fold the availability of resources of the control from the onset of stem elongation onwards), in which not only tillering was extended after the onset of stem elongation but also did not exhibit tiller mortality (Supplementary Fig. S1). In the control, each plant produced an average of slightly more than 4 shoots at the time of maximum number of tillers (c. 1000 shoots per m²) many of which died from then to anthesis (Supplementary Fig. S1). When the plants were shaded, the tiller death was similar to that of the control (Supplementary Fig. S1, the line fitted for the controls adjusted well to the data-points of both shading treatments); i.e., shading did not increase the mortality of tillers. On the other hand, thinning the plots at the onset of stem elongation clearly affected the dynamics of tillering and tiller mortality. The treatment Th50 mainly reduced the rate of tiller death resulting in an increased tiller survival (Supplementary Fig. S1), and in the case of Ascott in particular, tillering was slightly prolonged, and the maximum number of tillers was slightly increased as well (Supplementary Fig. S1). When 75 % of the plants were removed at the onset of stem elongation (Th75), tillering was prolonged increasing the maximum number of tillers, and eliminating tiller death (i.e., not only the maximum number of tillers increased slightly but also all the tillers that appeared survived and produced a spike). Therefore, the response to increases in availability of resources per plant during stem elongation was marginal and inconsistent (only in Th75) for the maximum number of tillers (Fig. 2A,C) and large and consistent for the tiller survival

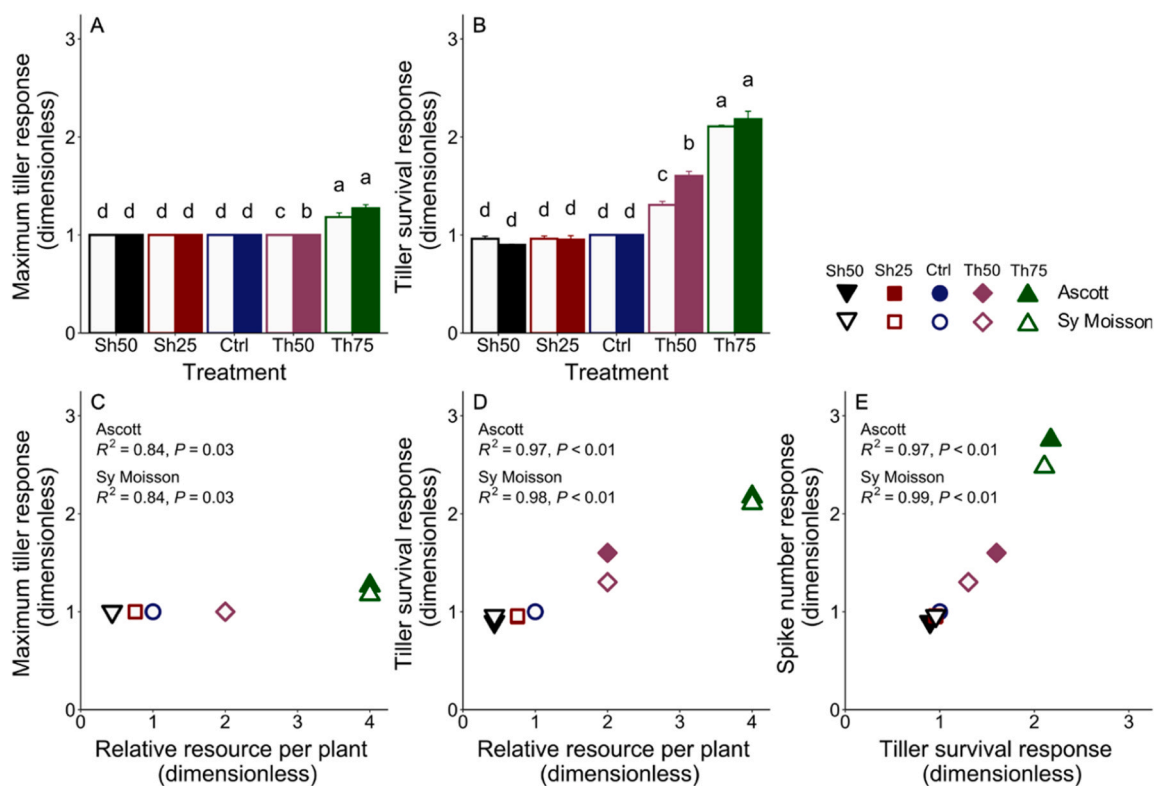


Fig. 2. Maximum number of tillers (A) and tiller survival (B) responses for each treatment, in Lleida. The error bars represent the standard error and different letters represent significant difference according to ANOVA and LSD test. Response to resource availability of the maximum number of tillers (C) and tiller survival (D), and the relationship between the responses of the final number of spikes and that of tiller survival (E) in Lleida. Inset the panels we included the coefficient of determination of the linear regression for each cultivar. Resource availability is expressed in relative terms to the control within each cultivar, which was fixed as 1. Open and closed symbols correspond to Sy Moisson and Ascott, respectively. Circles correspond to the controls, inverted triangles to shading 50 %, squares to shading 25 %, rhombuses to thinning 50 % and triangles to thinning 75 %.

(Fig. 2B,D). Neither the maximum number nor the survival of tillers responded to reductions in availability of resources per plant (Fig. 2A-D). Consequently, the response of the SN to the change in availability of resources was very strongly related to that of tiller survival (Fig. 2E).

3.2. Dynamics of spike growth and floret initiation-death, and spike fertility

The number of fertile florets per spikelet averaged across all the spikelets of the entire spike was very close to that considering only the basal, central, and apical spikelets (Supplementary Fig. S2). This implied that (i) the spikelets selected for the fine determinations of development (as described in Materials and Methods) did actually represent well the different spikelets of the spike, and (ii) the dynamics of floret development derived from averaging the three specific spikelets dissected would represent well the development of florets across the whole spike.

Even though the treatments were imposed during virtually the whole period of floret differentiation, neither the shading treatments delayed or reduced the rate of generation of florets nor the thinning treatments brought forward the process or accelerated the rate (Fig. 3A). Consequently, the maximum number of floret primordia generated, that was slightly higher in Ascott than in Sy Moisson, was negligibly affected by the shading or thinning treatments regardless of the genotype (Fig. 3A). On the other hand, the shading and thinning treatments noticeably affected the rate of floret death so that the number of fertile florets per spikelet at anthesis noticeably decreased with shading and increased with thinning (Fig. 3A). Simultaneously, the growth of the juvenile spike, which had been very small during floret initiation and became very large during the phase of floret death, also responded to the

resource availability treatments very similarly to the number of fertile florets (Fig. 3B). As a consequence of these responses, there was a positive relationship between the responses to treatments of the rates of floret primordia survival and of spike dry weight at anthesis (Fig. 4).

Although the details of the dynamics of floret initiation and mortality are available only for Lleida, in Clermont-Ferrand there was an assessment of the maximum number of floret primordia initiated and of fertile florets at anthesis in spikelets representative of the basal, central and apical positions of the spike. Alike in Lleida, the study carried out in Clermont-Ferrand found, consistently for both cultivars, that (i) the maximum number of floret primordia initiated was mostly unresponsive to the treatments (Fig. 5A); while (ii) the number of fertile florets at anthesis (Fig. 5B), was very consistently (in both cultivars in all spikelets) decreased by shading and increased by thinning (i.e., the more resources the higher the survival of primordia initiated and consequently the more fertile the spikes). Interestingly, even though there was a response in all three spikelet positions, they were moderate in apical and central spikelets but very strong in the basal spikelets, and this was also consistently found over the two locations and both cultivars, though more noticeably in Ascott (Fig. 5). This effect of treatments on number of fertile florets at anthesis was mainly related to the effects of treatments on floret primordia survival, particularly for the very large impact on the basal spikelets (Fig. 5C).

At anthesis, the number of fertile florets was counted in each of the spikelets across the spike in Lleida. Similar to what was observed in the few selected positions (apical, central, and basal), the variation in resource availability was positively associated with the shifts in the number of fertile florets consistently over the whole spike in both genotypes (Fig. 6). However, the variation was not in equal proportions among spikelets and depended on its position and on the genotype. The

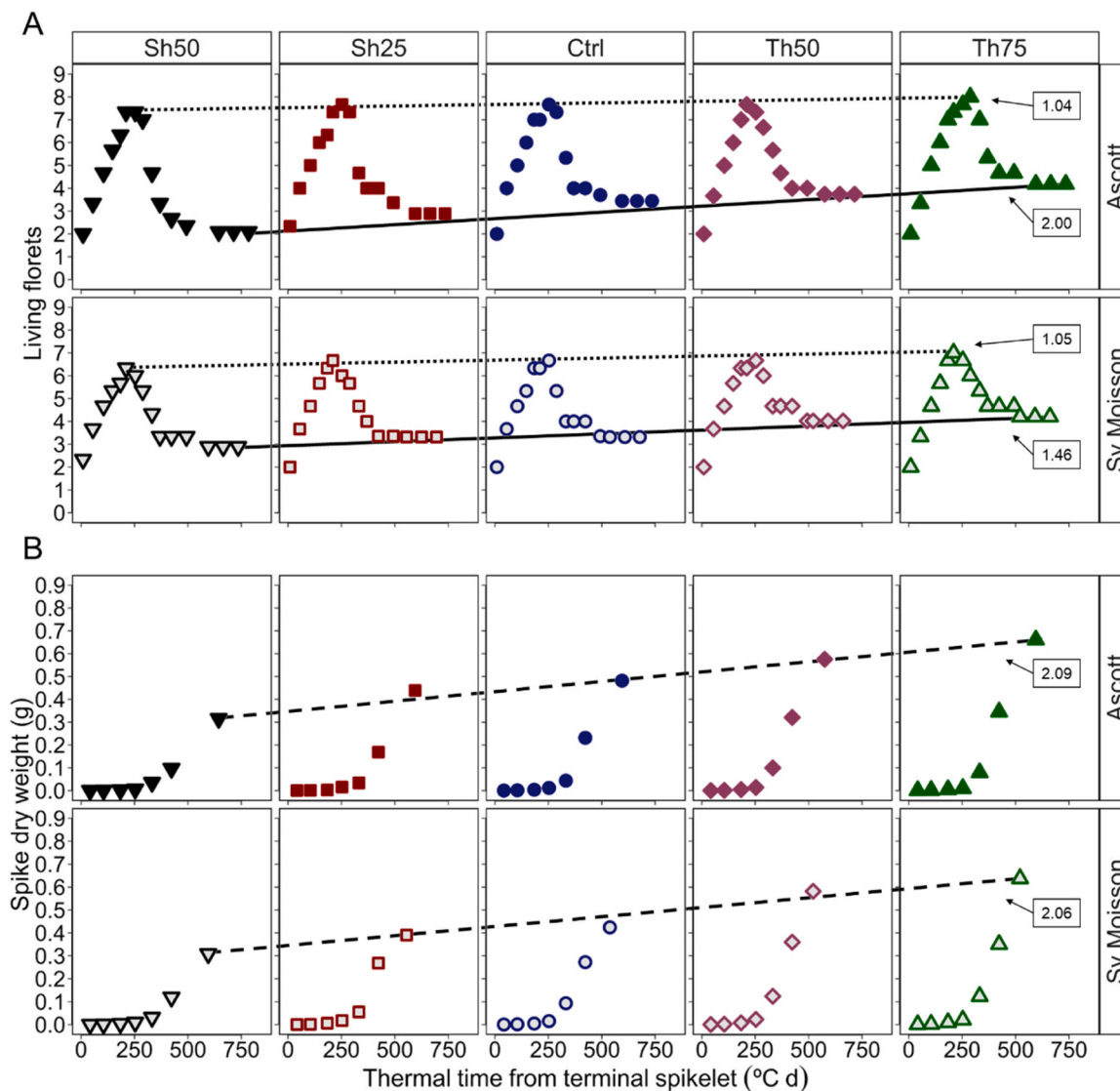


Fig. 3. Number of actively developing floret primordia (living florets, symbols as in Fig. 2 - averaged for different positions of the spike, A) and juvenile spike dry weight (B) through thermal time from terminal spikelet for each genotype-treatment in Lleida. The last data-point of each panel represents the values at anthesis of either the number of fertile florets per spikelet (A) or the spike dry weight (B). The white boxes connected by arrows indicate the Th75 to Sh50 ratio for maximum number of living florets (dotted lines), fertile florets at anthesis (solid lines), and spike dry weight (dashed lines), respectively.

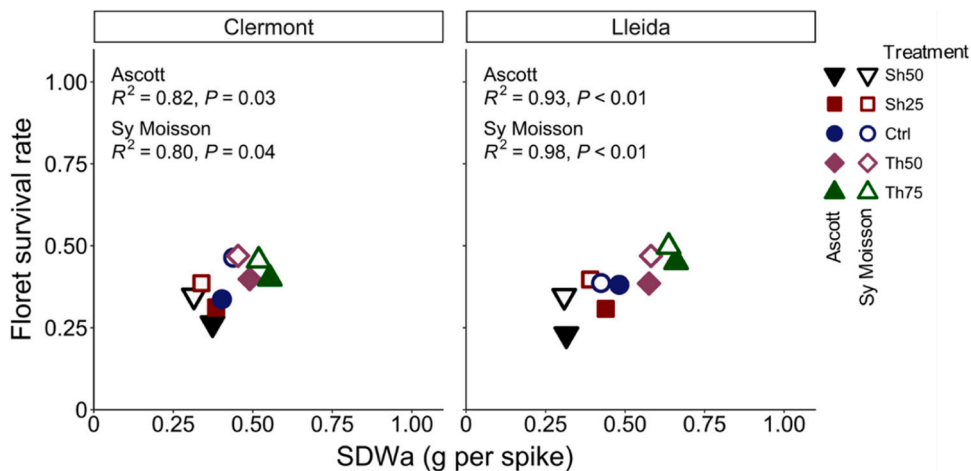


Fig. 4. Relationship between floret primordia survival rate (the ratio between final and maximum values) and spike dry weight at anthesis. Symbols as in Fig. 2 (open and closed symbols correspond to Sy Moisson and Ascott, respectively). Error bars do not appear as the standard error was in all cases smaller than symbols.

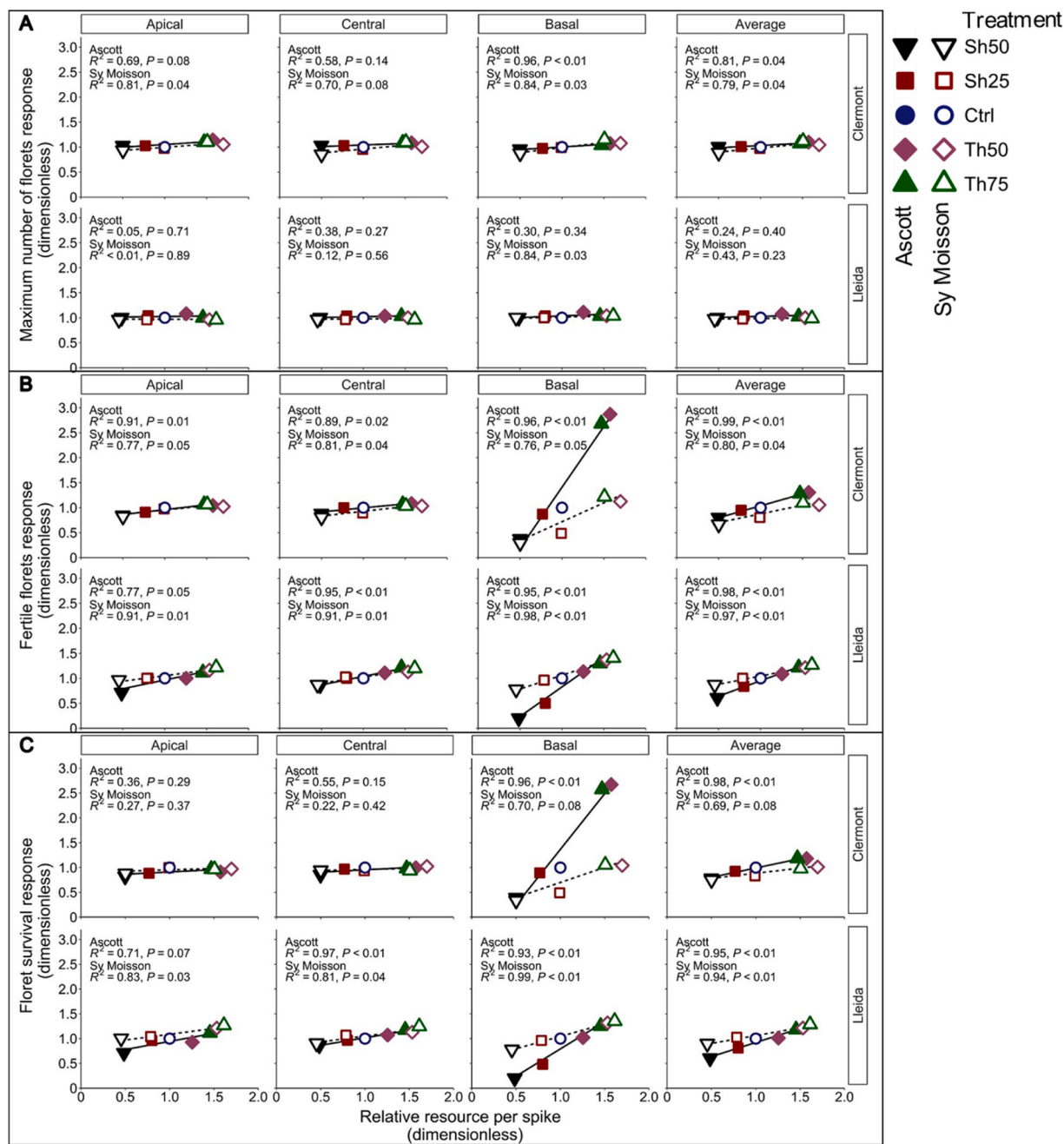


Fig. 5. Relationship between the responses of either the maximum number of floret primordia initiated (A), the number of fertile florets at anthesis (B), or the rate of floret primordia survival (C), and the relative resource per spike for each genotype-treatment in Clermont-Ferrand (top panels) and Lleida (bottom panels). Considering that unmanipulated plots received what would be the total amount of resources per area available to the crop in a given environment, its relative resource per spike was fixed in 1. The variable in the abscissa is dimensionless and represents the resource availability per spike according to the treatment compared to the control as well as the number of spikes m^{-2} a treatment had compared to its control for each location. Symbols as in Fig. 2.

overall response of Ascott to Sh50 was higher than that of Sy Moisson and the differences were mainly found at the basal portion of the spike (Fig. 6; Supplementary Fig. S3).

3.3. Development of individual floret primordia

The responses of spike fertility to the changes in availability of resources produced by the treatments were the consequence of the effects they had on the dynamics of development of particular floret primordia. Comparing these dynamics for each floret primordia that reached at least the stage of W3.5 between the extreme treatments (Sh50 and Th75; Fig. 7 and Supplementary Fig. S4) illustrates the impact of resource

availability on the likelihood of a particular floret to develop normally until becoming a fertile floret or die (the other treatments exhibited intermediate responses). For simplicity we illustrate the fate of the florets 1, 3 and 5 (Fig. 7) and the others (florets 2, 4 and 6) followed the same pattern (Supplementary Fig. S4). The rates of development of the different floret primordia was unaffected by the availability of resources during the initial phases (first 300–400°C d after terminal spikelet), and during the rest of the stem elongation period until anthesis the dynamics of floret development responded or not to the availability of resources depending on the particular spikelet and floret positions (Fig. 7 and Supplementary S4).

The floret primordium closest to the rachis (Floret #1) developed

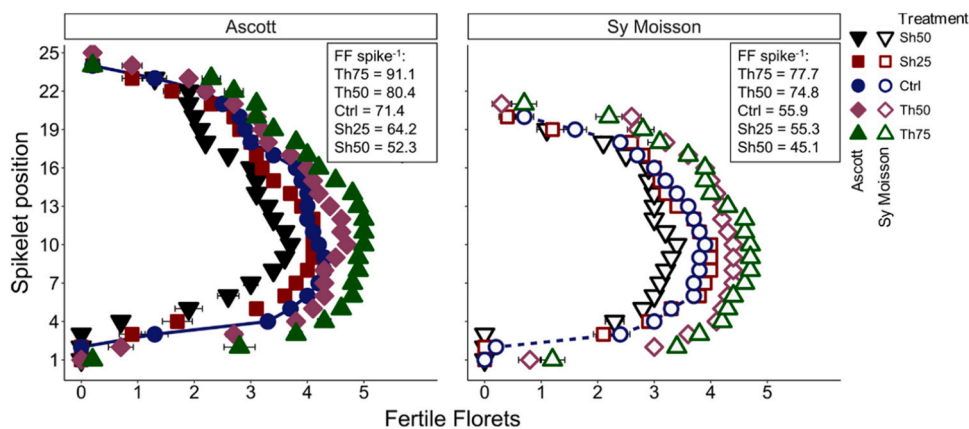


Fig. 6. Number of fertile florets for each spikelet of the spike at anthesis for each of the treatments with a line representing the control (Ascott, solid line and Sy Moisson, dashed line). Inset each panel the number of fertile florets per spike for each of these treatments is reported. Symbols as in Fig. 2. Error bars represent the standard error (not visible when smaller than symbols).

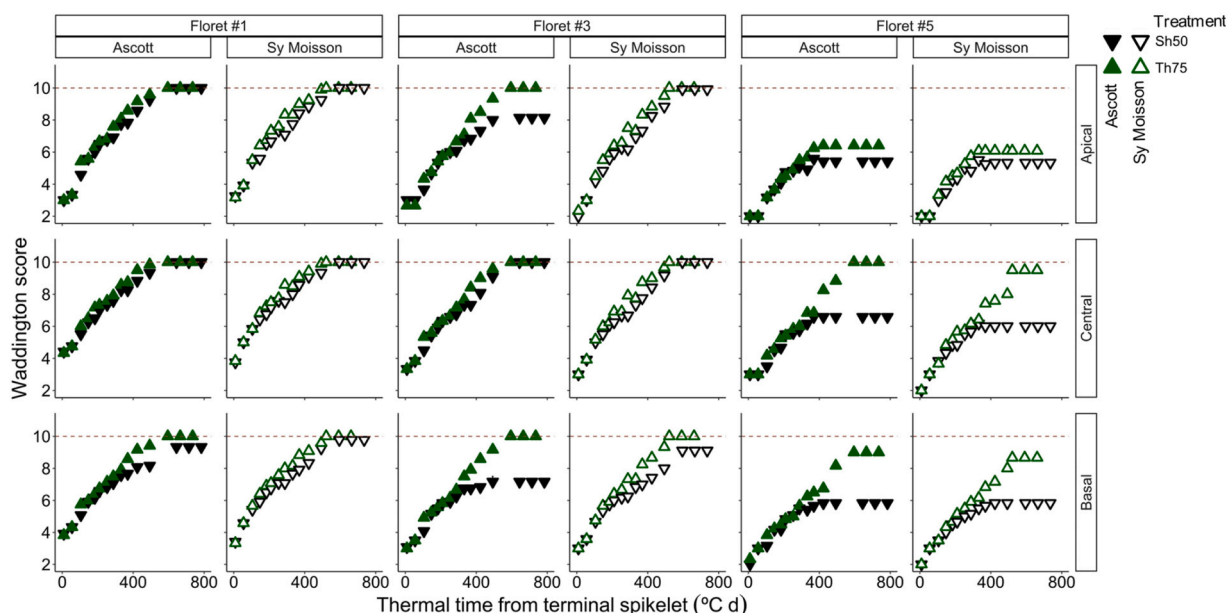


Fig. 7. Dynamics of floret development of florets 1, 3, and 5 in apical, central, and basal positions for Ascott and Sy Moisson in the most contrasting treatments (Sh50, inverted triangle and Th75 triangle) in Lleida. The stage of W10 corresponds to a fertile floret. Symbols as in Fig. 2. Error bars represent the standard error (not visible when smaller than symbols).

normally to become a fertile floret regardless of the treatment, except for the basal position in Ascott where even the floret primordium closest to the rachis did not reach fertility when shaded and that spikelet was sterile in all the plants inspected within that treatment (Fig. 7, panels in the first and second columns). For all other floret positions their fate depended on the particular spikelet position and treatment. In the central spikelets, floret 3 did also develop normally regardless of the availability of resources, but in the apical (in the case of Ascott) and basal spikelets (in both cultivars) the third floret primordium (Floret #3) developed normally in Th75 but died before becoming a fertile floret in Sh50 (Fig. 7, panels in the third and fourth columns). Floret 5 developed normally until becoming a fertile floret only in the central spikelet in Th75 but died at a relatively young stage of development in Sh50, and it died in apical and basal spikelets regardless of the availability of resources (Fig. 7, Floret #5). But even in these cases in which florets die in all treatments, the impact of the availability of resources was clear as the florets developed more before dying in Th75 than in Sh50 (Fig. 7). Thus, it was clear that the availability of resources played a central role in maintaining a normal rate of floret development for a longer period

determining in many, but not in all, cases an increase in spikelet fertility.

4. Discussion

In the companion paper (Bicego et al., 2024) we concluded that Ascott was constitutively more plastic, for both SN and GS, than Sy Moisson, and that the plasticity of GS was tightly related to that of FF. The different plasticity was not related to differences in phenology or yielding capacity: in both experiments Ascott and Sy Moisson demonstrated similar time to anthesis and yield under the control condition (averaging across locations they were 183 and 182 days to anthesis and 7.9 and 7.4 Mg ha⁻¹, respectively; Bicego et al., 2024). Here we intended to track down the mechanisms that determine these differences in SN and GS plasticity by analysing the dynamics initiation and death of tillers and floret primordia. This is because both SN and FF at anthesis are actually the result of processes determining a massive generation of structures (tillers and floret primordia) followed by a degeneration of many of the initiated structures (Kirby, 1988; Slafer et al., 2021). We found that for both tillers and floret primordia, survival was a key

contributor of the plasticity of the determinants of grain number components (SN and FF). The results shown in the present study cannot discard the relevance of tillering as the treatments were imposed at the onset of stem elongation, but they do highlight the relevance of structures survival to contribute to the plasticity of GN. This, indeed, agrees with both (i) previous research elucidating that the critical period for GN determination (Carrera et al., 2024) extends back to the end of late reproductive phase when the degeneration of structures take place (Fischer, 1985; Savin and Slafer, 1991; Slafer et al., 1994), and (ii) the evolutionary hypothesis that the energetic cost of overproducing reproductive structures (young tillers or floret primordia) at early stages, that will in great part be further degenerated to establish their final number, does not imply a disadvantage (Sadras, 2007; Sadras and Slafer, 2012; Stephenson, 1981).

Even though the treatments were applied at the onset of stem elongation that frequently coincides with the maximum number of tillers generated, they were further increased when the availability of resources increased dramatically in Th75. The fact that Th75 resulted in a prolonged tillering phase, that continued past the onset of stem elongation corroborates that the link between the end of tillering and the onset of rapid stem growth is not developmental but is dependent upon availability of resources (Alzueta et al., 2012; Xie et al., 2016). The Th75 not only resulted in a lengthened tillering period but also in an absence of tiller death. No such differences were observed in the generation of spikes when resources were restricted by shading or in the control in both locations. However, the genotypic differences in plasticity for this trait were given (i) in Th50 by a higher tiller survival of Ascott and (ii) in Th75, when resources were very abundant, by a slightly higher generation of tillers not followed by an increase in death. This indicates that Ascott presents a better use of resources dedicated to the survival of tillers. In other words the reason for the higher plasticity for SN in Ascott is its improved capacity to use resources more efficiently. Interestingly, when SN was increased, no resource seemed to be diverted from main stem to produce and keep tillers alive; indeed the main stems were heavier as resources increased (higher SDWa) and had higher spike fertility (more FF at anthesis).

Similarly to tillering, the different plasticity in fertile florets was mainly a consequence of the survival of initiated structures. However, the reduction of resources that had no effect on tiller number, did impact the floret development dynamics. Even though both shading treatments generated the same number of spikes as the control, they were not as fertile. The Sh50 had less fertile florets than Sh25, which in turn had less florets than control. Interestingly, even though the treatment application started soon after the initiation of the earliest florets of the central spikelets, there was no effect of resource availability on the generation of floret primordia. The maximum number of florets generated seemed to be somehow fixed and the number of fertile florets at anthesis was basically a consequence of higher or lower survival rates, which in turn have been proposed to be driven by the availability of resources (Dreccer et al., 2014; Ferrante et al., 2010; Miralles et al., 1998; Stockman et al., 1983). This is commensurated with the fact that the availability of resources at booting, when floret mortality starts to be very active (Kirby, 1998), is critical for determining floret survival (Dreccer et al., 2014; Serrago et al., 2008) and with the general view that the booting stage is critical for yield determination (Distelfeld et al., 2014; Guo et al., 2018; Ji et al., 2017; Fischer et al., 2024).

As expected, and in accordance with many studies (Calderini et al., 1997; Fischer, 1985, 2007), there was a positive relationship between SDWa and floret survival and consequently FF (in line with what has been consistently reported through time; e.g., Stockman et al., 1983; Miralles et al., 1998; Gonzalez et al., 2005). Interestingly, in both genotypes the SDWa was progressively higher as the resource availability increased but the responses to treatments were similar between genotypes while the FF response was higher in Ascott (Bicego et al., 2024). This could indicate that either, one genotype benefits more from more available resources, or suffers more penalty when resources are scarce,

while the other genotype shows more stability to changing available resources for this trait. The most plastic genotype (Ascott) had a higher fluctuation in survival rates when comparing the most contrasting treatments (Th75 and Sh50) and a more detailed analysis on the specific spikelet positions indicated that the higher differences were mainly found in the basal portion of the spike.

In our study, the most plastic genotype seems to take more advantage of the available resources by increasing the number of florets that survive overall the spikelets but even more noticeably in the basal spikelets that are usually sterile (e.g., Backhaus et al., 2023; Sun et al., 2024), but not constitutively sterile as they may become fertile if enough resources were allocated to the spikes (Tamagno et al., 2024). Likewise, when resources are scarce, this same portion of the spike suffers the strongest penalty in fertility. The basal spikelets of Ascott have higher variation in fertility compared to the other positions of the spike, and the reduced fertility is extended to positions closer to the centre as resources get more limited. That is, the low fertility of spikelets, when shading the plots, was not only observed in the few first spikelets (from bottom up) but it was extended to spikelets towards the centre of the spike. In contrast, with the high availability of resources in Th75 a higher fertility was observed in the same region of the spike. Previous study evaluating spike fertility of 10 wheat lines, found that the 2 most contrasting ones had a higher reduction in fertile florets at the basal spikelet (González-Navarro et al., 2015). This portion of the spike was also the most affected when shaded (Stockman et al., 1983; Slafer et al., 1994). In our study, this basal portion of the spike seemed to regulate the plasticity of spike fertility more than other positions. This is relevant as these basal spikelets tend to be sterile in most field conditions (Philipp et al., 2018; Yao et al., 2019). And it had been speculated that the sterility of the most basal spikelets of the wheat spike would be constitutive because of the delayed development of its florets compared to the central spikelets (Backhaus et al., 2023), however in our study both genotypes had at least one fertile floret in the very first spikelet with the Th75 treatment (occasionally in Ascott and consistently in Sy Moisson). Our results put forward the hypothesis that the most basal spikelets that are effectively sterile in normal agronomic conditions are so because lack of enough resources being allocated to the growing spike (Tamagno et al., 2024).

For most genotypes, 3–4 grains seem to be a maximum even in the central spikelets (e.g., Ferrante et al., 2013a), likely due to physical constraints. Therefore, any increase in the availability of resources that allows an increase in grain set in the spike is likely to be allocated to new grains in a proximal position in an extreme spikelet, rather than an extremely distal grain in a central spikelet. In other words, in case of an energetic surplus, florets of the basal portion of the spike that are usually not the priority, could also survive and reach fertility. Alike, when plots were shaded, available resources are allocated to the central position of the spike but are not sufficient to basal positions to reach fertility. From an energetic point of view, investing in generating floret primordia does not represent a significant cost (as discussed in Sadras and Slafer, 2012). Nevertheless, it seems pointless to set a fertile floret in a position that has a low probability to complete its cycle if there are still positions that are safer to invest in (central ones). The source limitation at the late reproductive phase seems to regulate the survival of the florets and the spike fertility in more distal positions may be linked to the amount of source available in that phase.

5. Conclusion

Intuitively, variations in availability of resources would bring direct variations in yield and its components. This is true for nutrients and water, and with some similarity for crops facing other abiotic or biotic stresses. However, the genotypic response to those changes may vary and understanding the physiological basis of the plasticity of the main components of grain number may be useful. In this study, we found that manipulating resource availability at the onset of stem elongation

mainly affected the survival of the generated structures. Regarding genotypic differences in spike fertility plasticity, the responses appeared to be primarily related to the basal portion of the spike.

CRedit authorship contribution statement

Gustavo A. Slafer: Writing – review & editing, Visualization, Supervision, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Breno Bicego:** Writing – original draft, Visualization, Methodology, Formal analysis, Data curation. **Roxana Savin:** Writing – review & editing, Supervision, Investigation, Formal analysis, Data curation. **Christine Girousse:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Vincent Allard:** Writing – review & editing, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.fcr.2024.109654](https://doi.org/10.1016/j.fcr.2024.109654).

Data Availability

Data will be made available on request.

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