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Plasticity of grain number and its components in contrasting wheat cultivars

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ABSTRACT

Context: A better understanding of the plasticity of grain number (GN) components may be essential to design better management and breeding strategies. Alterations in resource availability during pre-anthesis may affect the interplay between the main GN components, spike number (SN) and grains per spike (GS). Quantifying the magnitude, and understanding the physiological bases, of differences in plasticity of GN components within elite material would be valuable for breeding.

Objective: The aim of this study was to investigate the physiology of GN determination, the plasticity of, and any possible trade-offs between its components.

Methods: Two modern genotypes (Ascott and Sy Moisson), expected to have contrasting GS response to changes in SN, were subjected to 5 levels of resource availability (two levels of shading, two levels of thinning and an unmanipulated control) from the onset of stem elongation to anthesis in two locations (France and Spain).

Results and conclusions: Yield in the control was relatively high in both locations and strongly related to GN, which was more plastic in Ascott than in Sy Moisson; and the difference in plasticity was related to different responses of the two main GN components. SN acted as a coarse regulator of yield contributing with crop level plasticity while GS acted as a fine-tuning mechanism expressing the smaller responses to resource at genotypic level. The higher GS plasticity of Ascott-like genotypes may provide more stability in early stress scenarios where the SN is reduced as long as no later limitations occur.

Implications: The acceptance of a hypothesis on genotypic variation in plasticity of GS derived from multilocation and multiyear datasets, and the consistency of the genotypic difference in response to changes in resources across the two locations, suggests that the genotypic difference in plasticity may be constitutive and would therefore be useful in designing strategic crosses aiming to combine plasticity of GS with other yield-related attributes.

1. Introduction

Expected population increase implies a greater demand for food whose production is not currently increasing at the required pace (Ray et al., 2013; Tilman et al., 2011). Wheat is one of the most widely grown crops (Slafer et al., 2021) and is already critical for food security (Reynolds et al., 2012). The challenge of increasing wheat productivity is even greater considering that it must be mainly achieved through increasing wheat yield (Slafer et al., 2022, and references quoted therein), due to environmental and socio-economic limitations to significantly expand the growing area or using more inputs (Bruinsma,

2009; Lal, 2016). The improved productivity shall be reached by increasing both yield potential and tolerance to stresses. As both are rather complex, their improvement would be more likely achieved if we gain more insight into their physiological determination (Araus et al., 2008; Foulkes et al., 2011; Reynolds et al., 2022). In particular, they would depend on the exploitation of plasticity of yield determinants; i. e., the amount by which a trait in a particular genotype is changed in response to changes in environment (Bradshaw, 1965). The phenotypic plasticity for determinants of yield would be instrumental for improving adaptation to different environments (Peltonen-Sainio et al., 2011; Sadras et al., 2009), and has been used to understand the relationships

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between yield components based on evolutionary and breeding constraints (Sadras and Rebetzke, 2013).

As wheat normally has a rather high source-to-sink ratio during the effective period of grain filling (Borrill et al., 2015; Reynolds et al., 2005; Serrago et al., 2013; Slafer et al., 2023a) yield is far more related to the number than to the average weight of the grains (Peltonen-Sainio et al., 2007; Sadras, 2007), and grain number per m^2 (GN) is far more plastic than grain weight (Sadras and Slafer, 2012; Slafer et al., 2014). Therefore, what seems critical is to understand the physiology of grain number plasticity which would require the understanding of the plasticity of the components of GN: the numbers of spikes per m^2 (SN) and of grains per spike (GS). SN results from generation, development, and survival of tillers (Dreccer et al., 2013; Slafer et al., 2009); and GS involves floret initiation and survival within each of the developing spikes (Ghiglione et al., 2008; González et al., 2011) and grain setting (Ferrante et al., 2013). In wheat, the phase of tiller and floret mortality are critical for spikes and GS, respectively (Slafer et al., 2021). As the dynamics of generation and degeneration of structures determining *a posteriori* SN and GS overlap in time (Slafer and Rawson, 1994), a feedback control between them can be expected when the crop is exposed to more or less resources (Reynolds et al., 2022). In other words, available resources allocated to one of these components may be detracted from (or at least may not be made available to) the other. As the material and energy requirements for survival of one reproductive tiller is larger than those for survival of a microscopic floret primordium, it can be expected that, when determining GN during stem elongation, SN would act as a coarse-regulation mechanism (Sadras and Rebetzke, 2013; Sadras and Slafer, 2012; Zhang et al., 2010) whilst GS would mainly be a fine-tuning mechanism. Therefore, both components might respond to modest changes in environmental drivers of yield, but SN is more likely to accommodate large changes (Slafer et al., 2022). Within the genetic variability available in elite germplasm[1] there is variation in the degree of plasticity for SN and GS (Slafer et al., 2022). Quantifying the magnitude, and understanding the physiological bases, of differences in plasticity of these components within elite material would be valuable for breeding. For instance, when aiming to improve yield potential breeders may prefer to use parents of high plasticity maximizing yield in high-yielding conditions, even though counting with a potential higher risk of yield reduction in less favorable ones. On the other hand, if aiming to improve resilience to stressful conditions, less plastic parents might confer that stability to the progeny.

Recently, the screening of a large wheat genetic panel done in a multilocal trial with 30 environments (location x year x management) in France evidenced a wide range of genetic variation in plasticity of SN and GS. Among all 240 genotypes tested, we selected two modern cultivars, Ascott and Sy Moisson as examples of high-yielding genotypes having contrasting levels of plasticity of GS in response to changes in SN, but having very similar phenology (and then their different plasticity of GS would have been independent of differences in time to flowering).

Although the ensembled analysis of multilocal experiments are relevant to propose hypothetical genotypic differences in plasticity, rigorously testing the hypothetical behaviors in experiments directly manipulating the availability of resources for SN and GS determination is critical to reach solid conclusions on plasticity as well as to study the mechanisms related to them. Therefore, the aim of this study was to investigate the physiology of GN determination and plasticity of its components in two modern wheat cultivars with hypothetical differences in plasticity of GS to changes in SN. For that purpose, we determined yield and yield components, plasticity of SN and GS, and any

possible trade-offs between components. In a companion paper, we analyzed the dynamics of generation and degeneration of structures responsible for the responses of SN and GS to the changes in availability of resources.

2. Materials and methods

2.1. General conditions

Two sister experiments were conducted during the 2020–21 growing season in Spain and France. The experiment in Spain was sown close to Bell-Lloc d'Urgell (41°36′46.4″N, 0°48′19.7″E) in the province of Lleida (NE Spain) in a calcaric fluvisol soil (ESDB, 2004). The experiment was sown on 24 November 2020 at a rate of 350 seeds m^{-2} and soon after seedling emergence the plots were manually thinned to have a final density of 250 plants m^{-2} uniformly distributed. The experiment in France was sown at Clermont-Ferrand (45°46′37.0″N, 3°8′30.2″E) in a vertic fluvisol soil (ESDB, 2004). The sowing date was 06 November 2020 at a rate of 190 plants m^{-2}. In Lleida, the plots consisted of 6 rows, 0.2 m apart and 4 m long while in Clermont, they were 8 rows, 0.17 m apart and 5 m long.

The Bell-Lloc experimental field had alfalfa as previous crop and the soil analysis taken up to 1 m depth resulted in an average available N content of 342 ± 5.6 kg of N ha^{-1} and therefore the experiment was not fertilized. The average available N content of the experimental area in Clermont was 174 kg of N ha^{-1} and plots were fertilized with 50 kg of N ha^{-1} uniformly applied to each plot at Z29 (end of tillering). In both experiments, weeds, diseases and pests were prevented or controlled by spraying recommended products at the doses suggested by their manufacturers.

Supplemental irrigation was applied in both locations to avoid water stress. In Lleida, the experimental field was flood irrigated twice during the growing season with approximately 60 mm on each occasion (Fig. 1). At Clermont the experimental field was irrigated twice with 30 mm on each occasion using sprinklers.

Meteorological data for each experimental field were recorded from the meteorological station of Meteocat (Servei Meteorològic de Catalunya) in Bell-lloc and from the nearby Aulnat weather station (Météo-France) in Clermont (Fig. 1).

2.2. Treatments and experimental design

Treatments consisted in a factorial combination of two contrasting genotypes regarding their plasticity in GS to changes in SN (Ascott, Limagrain Europe, 2012 and Sy Moisson, Syngenta, 2012) and five levels of resource availability (two reductions, two increases and an unmanipulated control) arranged in a completely randomized block design with three replicates in both locations.

The wheat genotypes were selected based on a large screen done by Arvalis, a french technical institute, in association with French private breeders, across many environments (a large set of multilocation x multiyear trials including virtually all commercial French cultivars). Among all the commercial cultivars considered, Ascott and Sy Moisson expressed the largest differences in the slope of the relation between grain number per spike and spike number per m^2 while displaying close mean spike number per m^2 and yield across environments.

The resources were decreased or increased at the onset of stem elongation (stage DC 31 of the decimal code scale of Zadoks et al., 1974), to minimize any effects of the shading and thinning treatments on the tillering and spikelet initiation processes to focus on their effects on tiller mortality and floret development within spikelets as major determinant of SN and GS, respectively. The reduction consisted of two levels of shading from the onset of stem elongation to anthesis (DC65) (Fig. 2). Shading was produced by cloths covering the canopy of the experimental plots that reduced the incoming radiation by 25 (Sh25) and 50 % (Sh50). For this purpose, in Lleida we installed over the designated plots

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[1] the gene pool chiefly exploited by breeders when aiming to improve complex traits, such as yield potential and resilience to stress (Allier et al., 2020; Rasmusson and Phillips, 1997; Rattey et al., 2009), because a wide cross would likely penalize severely the superior agronomic behavior(Kannenberg and Falk, 1995), that is fixed in the elite germplasm.
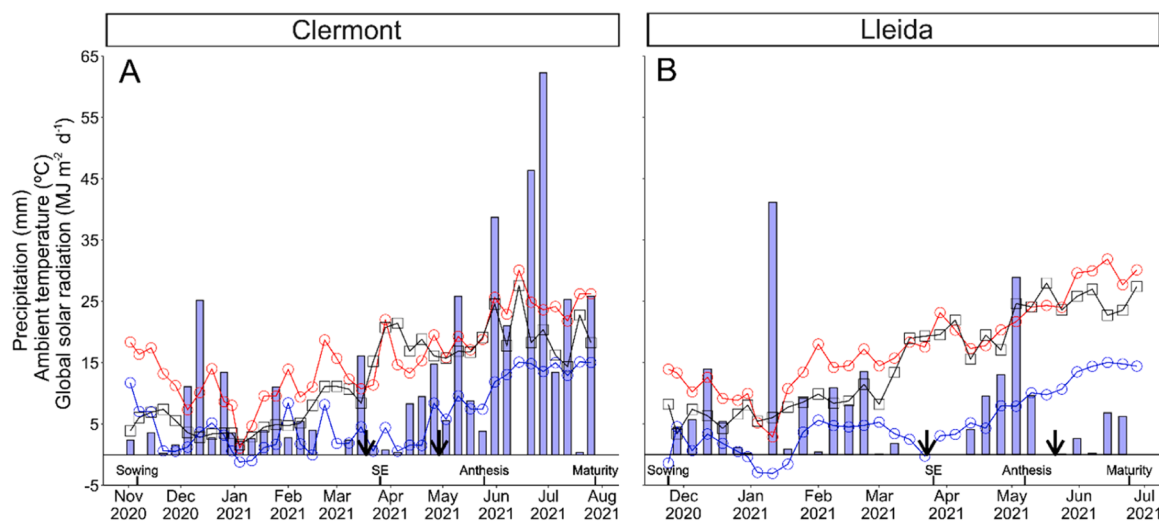
Fig. 1. Weekly averages of ambient maximum and minimum temperatures (circles) and global radiation (squares); and precipitation accumulated per week (bars) across the growing season in Clermont (A) and in Lleida (B). Downward arrows in the top panel represent the two irrigations (60 mm each). Developmental stages (onset of stem elongation [SE], anthesis, and maturity) are indicated in the bottom.

wood structures (1 m height, 1.2 m width and 4 m long) above the whole plot covered with the selected cloth. In Clermont-Ferrand the structures were 3 m long thus covering 60 % of the plot surface. The part of the plots used for experimental work were arranged in a staggered formation to minimize interaction between adjacent plots. Furthermore, the interference from the shading structures on neighboring plots was minimal because it only affected plots on one side or the other, either early in the morning or late in the afternoon when radiation levels were low. Moreover, it primarily impacted border rows, which we did not sample from. Shading did only marginally affect the temperature in the Sh50 treatment. Hourly temperatures were determined inside the Sh25 and Sh50 plots and in the control, with sensors placed in the central portion of the plot at c. 0.8 m height. The overall average temperature during stem elongation was 13.3 and 13.1°C in the control and Sh50, respectively in Lleida and 11.2 and 11.1°C in Clermont.

The increments in resources per remaining plant were produced by thinning the plots potentially reducing competition by 50 (Th50) and 75 % (Th75) (Fig. 2). As the experiments were irrigated and fertilized, thinning is expected to mainly reduce the competition for light increasing photosynthesis and growth of the remaining plants (Fischer and Laing, 1976).

Th50 was imposed through removing every other row of the experimental unit and Th75 through an additional removal of every other plant within the remaining rows. Therefore, the treatments Sh50, Sh25, Th50, and Th75 broadly provided 0.5-, 0.75-, 2-, and 4-fold the resources available to the plants relative to the control (1-fold), respectively (Fig. 2). Then, the term resources per plant will be used when presenting the results of different variables. As the plots were irrigated and fertilized and free of biotic stresses, the differences in resources mainly refer to differences in radiation per plant.[2]

2.3. Measurements and analyses

In Lleida, samples of aboveground biomass were taken at anthesis (DC 65) and at maturity (DC 90) from each experimental unit from a

sample area of 1-m long of a central row, which had been labelled shortly after seedling emergence. Thus, ensuring that the plant density and uniformity was as expected and that the interplant variability within the sample was minimized. In Clermont, 50 consecutive spikes were collected at maturity (DC 90) from the center row of each experimental unit.

The number of spikes was determined from counting them in the sample (and then upscaled to the m²). Then, we separated the main shoot and tiller spikes of the sample, threshed the spikes to obtain the number of grains and weighed them after oven drying them for 48 h at 65°C. The number of grains per spike and the average weight of the grains were calculated from these determinations. In Lleida, the sample at anthesis was processed as that of maturity and before oven drying the spikes, the number of fertile florets was counted in a subsample of 3 main shoot spikes, opening each spikelet and counting fertile florets. We considered a fertile floret any floret primordium that was in W10 (Waddington et al., 1983), or sufficiently developed to be irreversibly committed to become a fertile floret (W9); i.e. any floret with style and stigmatic branches spreading and green or yellow anthers visible (Ochagavía et al., 2021; Zhang et al., 2021).

Results are presented on a per plant basis: as the plots were true crops the measurements were made on a crop basis and values divided by the number of plants in each treatment for facilitating the viewing of the treatment effects. In addition, the traits responses for each treatment were calculated as relative values respect to the control within each genotype. That is, when reporting results as responses, the control plot was fixed as the unity and the response of a particular resource availability treatment was the ratio of its absolute value and that of the control within each cultivar. We considered the plasticity of the traits considered for each genotype to the norm of reaction when regressing the relative response of the trait to the relative resource availability, in both cases relative to the control (for each genotype within each experiment).

To analyze the effects of treatments, analyses of variance (ANOVA) were performed followed by *a posteriori* test using the contrasts method and linear regression were performed to determine the degree of relationship among variables. All statistical analyses were carried out with R statistical software version 4.3.0 (R Core Team, 2023).

3. Results

Time to anthesis was longer in Clermont than| in Lleida

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[2] Please note that this is a very broad assumption made to have a sound approach to a quantitative description of the variation in availability of resources, allowing us to estimate plasticity as the slope of the linear relationship between the responses of the traits and the relative availability of resources. Most likely both the reductions (shading) and the increases (thinning) in availability of resources are overestimated (see Discussion).
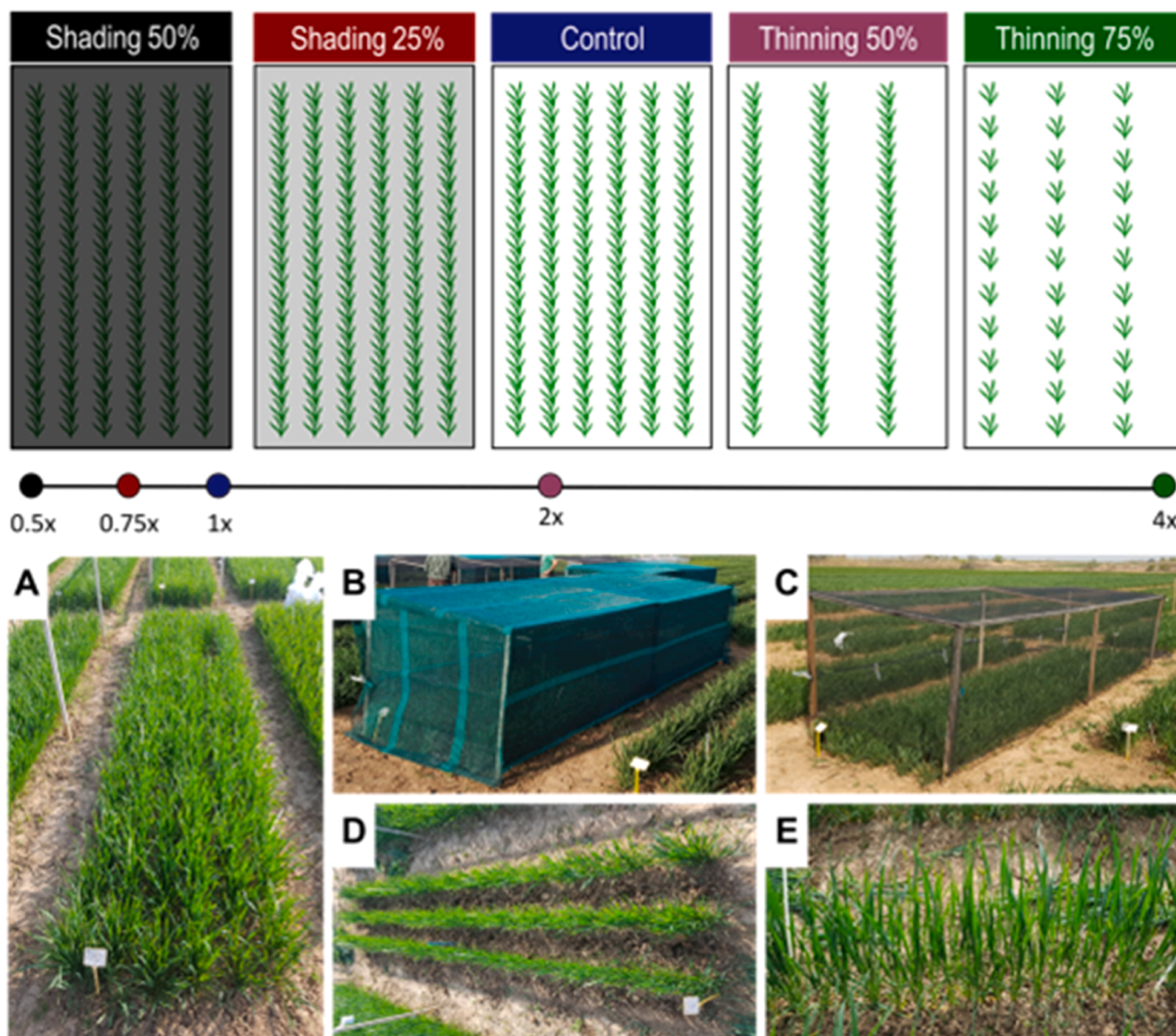
Fig. 2. Scheme representing the imposed treatments and control plots (top) and pictures of the plots when these treatments were imposed (bottom). In the top panel the scheme is presented along with below the resource availability scale that represents the relative resource availability compared to the unmanipulated plots. The Sh50 and Sh25 had 0.5- and 0.75-fold of the available radiation compared to the control, respectively. Likewise, the Th50 and Th75 had 2- and 4-fold the availability of resources compared to the control, respectively. The pictures in the bottom panel show the unmanipulated control (A), and the treatments of shading 50 % (B), shading 25 % (C), thinning 50 % (D), and thinning 75 % (E) immediately after they were imposed (at the onset of stem elongation).

(Supplementary Fig. S1A), which is expected for a location in N France when compared to one in the Mediterranean Basin. Yield in the control treatment was relatively high in both locations. Averaging across both genotypes, yield was 6.67±0.40 and 8.56±0.32 Mg ha^{-1} (equivalent to 3.5±0.2 and 3.4±0.1 g plant^{-1}) in Clermont and in Lleida, respectively (Supplementary Fig. S1C). As expected, when selecting them, the two cultivars had very similar time to anthesis and yield in the control treatment (Supplementary Fig. S1B, D). The similar phenology is strongly evidenced as the box-plot including the genotypic variation in time to anthesis for each of the two contrasting locations exhibited a negligible variation (Supplementary Fig. S1A) and the box plots for each cultivar were almost overlapped, both with large variation due to the differences between locations (Supplementary Fig. S1B). Averaging across locations, yield of Ascott and Sy Moisson was 7.88±0.45 and 7.35 ±0.62 Mg ha^{-1} (equivalent to 3.59±0.04 and 3.35±0.23 g plant^{-1}), respectively (Supplementary Fig. S1D).

Naturally, the increase and the reduction of resource availability per plant resulted in higher and lower GY compared to the unmanipulated control, respectively in both locations and genotypes (Fig. 3). Not surprisingly, given the relative intensity of treatments, the magnitude of gains when increasing resources with thinning (2- to 4-fold that of the control) was much higher than the penalty from reducing incoming radiation by 25 and 50 % with shading (Fig. 3).

Grain yield and grain number had a strong and positive linear relationship across genotypes and locations (R^2 > 0.97 and p-value < 0.001, Fig. 3). Indeed, grain number responded linearly to the resource availability of the different treatments (Fig. 4). Interestingly, although the response trends were similar for both genotypes, and consistent across the two locations, they were not identical in magnitude. Ascott tended to show a stronger response than Sy Moisson in both locations (Fig. 4A,B). To quantify the difference in plasticity between the cultivars, we regressed the response of grain number per plant against relative resource availability (the norm of reaction being the actual estimate of plasticity, see above). In both locations, Ascott exhibited a higher slope
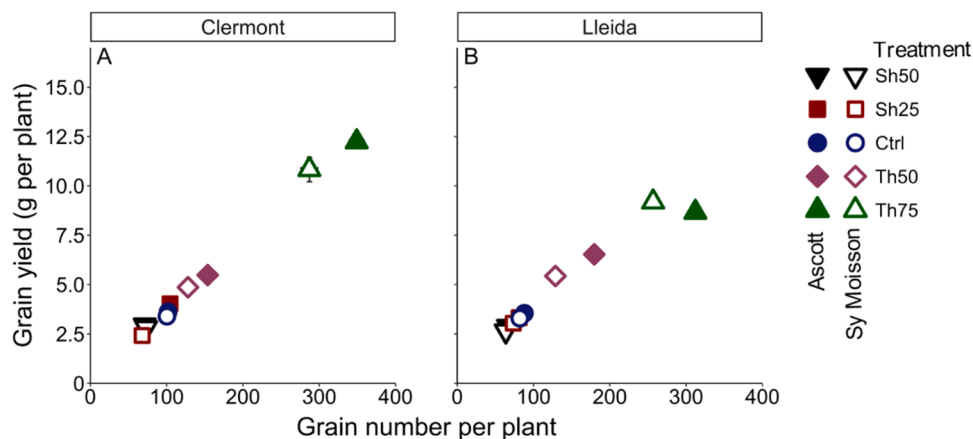
Fig. 3. Relationship between grain yield and grain number for each genotype-treatment in Clermont (A) and Lleida (B). Open and closed symbols correspond to Sy Moisson and Ascott, respectively. Circles correspond to the controls, inverted triangles to shading 50 %, squares to shading 25 %, rhombuses to thinning 50 % y triangles to thinning 75 %.
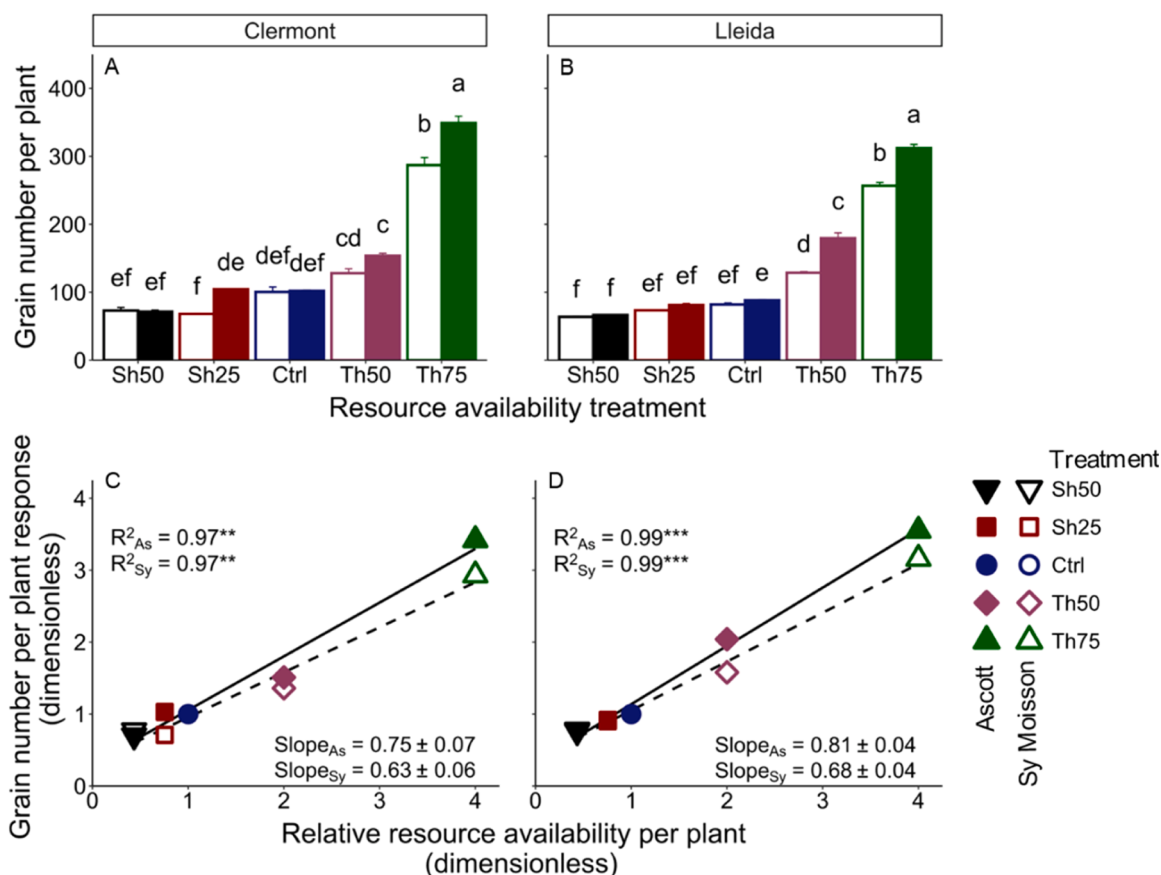

Fig. 4. The upper panels represent the grain number per plant of Ascott (solid bars) and Sy Moisson (open bars) for each treatment in Clermont (A) and Lleida (B). The error bars represent the standard error and different letters represent significant difference according to ANOVA's LSD for each location. The lower panels represent the relationship between grain number response (i.e., the treatment value relative to the control within each genotype) and resource availability for each genotype-treatment in Clermont (C) and Lleida (D) along with the linear regression parameters (R^2 and slope ± standard error) for Ascott (As, solid lines) and Sy Moisson (Sy, dashed lines). Resource availability is expressed in relative terms to the control, which was fixed as 1, on each location as represented in the scale of Fig. 2. Symbols as in Fig. 3.

than Sy Moisson, though the difference was more clear in Lleida (Fig. 4B, D). We presented the results for each location independently to underscore the consistency in the genotypic response differences. However, pooling the data from both locations for each cultivar highlights their differing plasticity more robustly (Supplementary Fig. S2).

The fact that the slopes are all lower than 1 and the intercepts higher

than 0, reflects that our choice to approximate the quantitative variation of the resource availability in each modality by the quantitative changes in shading and thinning is not perfect. Nevertheless, this approximation appears to us to be relevant to estimate plasticity differences between cultivars.

Even though GN responded positively to the resource increments

which was reflected in GY increments in both locations for both genotypes, there was an inconsistent negative response of AGW to increase of resources, in Lleida (Supplementary Fig. S3). However, the decline in AGW when increasing the availability of resources was very slight compared to the magnitude of the response of grain number per plant and therefore it did not represent a relevant trade-off.

3.1. Spike number response to resource availability

Grain number per plant was linearly related ($R^2 > 0.99$ and p-value < 0.001) to spike number per plant across the resource availability levels (Fig. 5). When drastically modifying the availability of resources during stem elongation, spike number per plant clearly responded to thinning, while the response to shading was not clear (Fig. 6). These differential responses to thinning and shading were consistent across the genotypes and locations. The number of spikes per plant under control condition was similar between genotypes for a given location: c. 2.5 in and c. 1.9 in Clermont and Lleida respectively (Fig. 5). Comparing Th75 to the control, in Clermont there was a 2.74-fold and 2.67-fold increase in the number of spikes per plant for Ascott and Sy Moisson, respectively, and the differences in response between genotypes was slightly higher in Lleida: 2.75- and 2.49-fold increase in Ascott and Sy Moisson, respectively (Fig. 6).

The shading treatment did not affect the plant population (there was no plant mortality during stem elongation due to the shading) and the response of SN was not noticeable. Then, the reduction in resources per plant did not result in an increase in tiller mortality. We can note that this response is opposite to the response to thinning that increased tiller survival very noticeably and consistently (Fig. 6).

3.2. Grain number per spike response to resource availability

The trend in GS response to availability of resource per plant was similar between the locations, however they were not as clear as expected, most likely because the large plasticity exhibited by SN to the increase in resources generated by Th75 (Fig. 6). In general, in both locations, there was a clear and more or less linear GS response resources available per plant from Sh50 to Th50 but the increment from Th50 to Th75 was only marginal and non-significant (Fig. 7). However, the fact that GS did not decrease when SN increased noticeably may reflect the use of additional resources in setting grains within the spikes: increasing SN by 2.5-fold or more, that had increased the proportion of late and potentially smaller spikes, did not decrease the average GS. Consistently across resource treatments and locations, Ascott had spikes with higher fertility than Sy Moisson (Fig. 8).

The lack of further increases in GS when comparing Th75 *vs* Th50

treatments may well be reflecting the fact that the large response in tiller survival (see above) led to a marginal increase in the relative resources per spike. Indeed, when analyzing the GS in response to the relative resources per spike the relationships are consistently linear (Fig. 9), i.e. there was a clear trend for GS to reflect the amount of resources available for each growing spike within surviving tillers, with data-points of Ascott on top of those of Sy Moisson, reflecting the constitutively larger GS of the former cultivar. But beyond the constitutive differences in GS, this component of grain number tended to be more plastic in Ascott than in Sy Moisson (Fig. 9).

As the responses were similar across all spikes of the crop (Supplementary Fig. S4), the main shoot spikes can be considered as representative of the whole spike population. As a consequence, GS response to resource availability per spike are similar when observed on main shoot only (Fig. 10) or on the whole spike population (Fig. 9). Ascott again exhibited more plasticity than Sy Moisson when analyzing the main shoot spikes separately (Fig. 10).

There were differences in number of spikelets per spike between genotypes, but expectedly no treatment effect on this trait (Supplementary Fig. S5). Ascott tended to have more spikelets compared to Sy Moisson (22.6±0.08 *vs* 19.3±0.1, Supplementary Fig. S5), being that the reason for the constitutive higher spike fertility. The number of grains per spikelet responded to the treatments (Supplementary Fig. S6). There was also a positive response to the increase in resources. Interestingly, Ascott did not show less grains per spikelet than Sy Moisson even when having consistently higher number of spikelets per spike (Supplementary Fig. S6).

3.3. Fertile florets and spike dry weight at anthesis

In Lleida, detailed anthesis data were collected. The GS was directly related to the number of fertile florets per spike at anthesis (FF; Fig. 11A) and, likewise, responded to the increments of resources allocated to the spikes (Fig. 11B). Expectedly, GS was lower than FF, however we did not observe any clear treatment effect on grain setting. Alike GS, FF plasticity in Ascott was higher than in Sy Moisson. At anthesis, the spike dry weight (SDWa) already responded to all treatments including both thinning modalities. There was a direct relationship between FF and SDWa and again Ascott tended to be more responsive than Sy Moisson.

4. Discussion

An overall view of the results confirmed well-established findings in the literature (see Slafer et al., 2023a, and many references therein): yield responses to resource availability during stem elongation are substantial and are mainly driven by responses of grain number. This
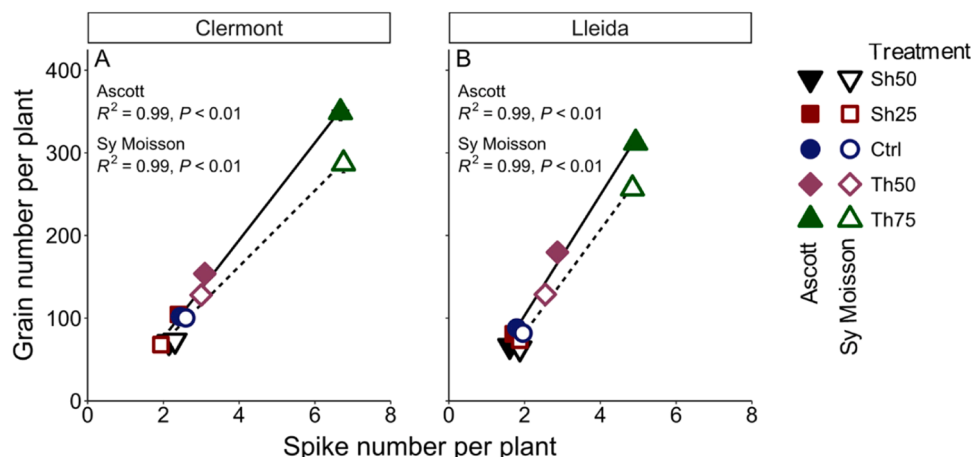

Fig. 5. Relationship between grain number and spike number per plant for each genotype-treatment in Clermont (A) and Lleida (B). Symbols as Fig. 3.
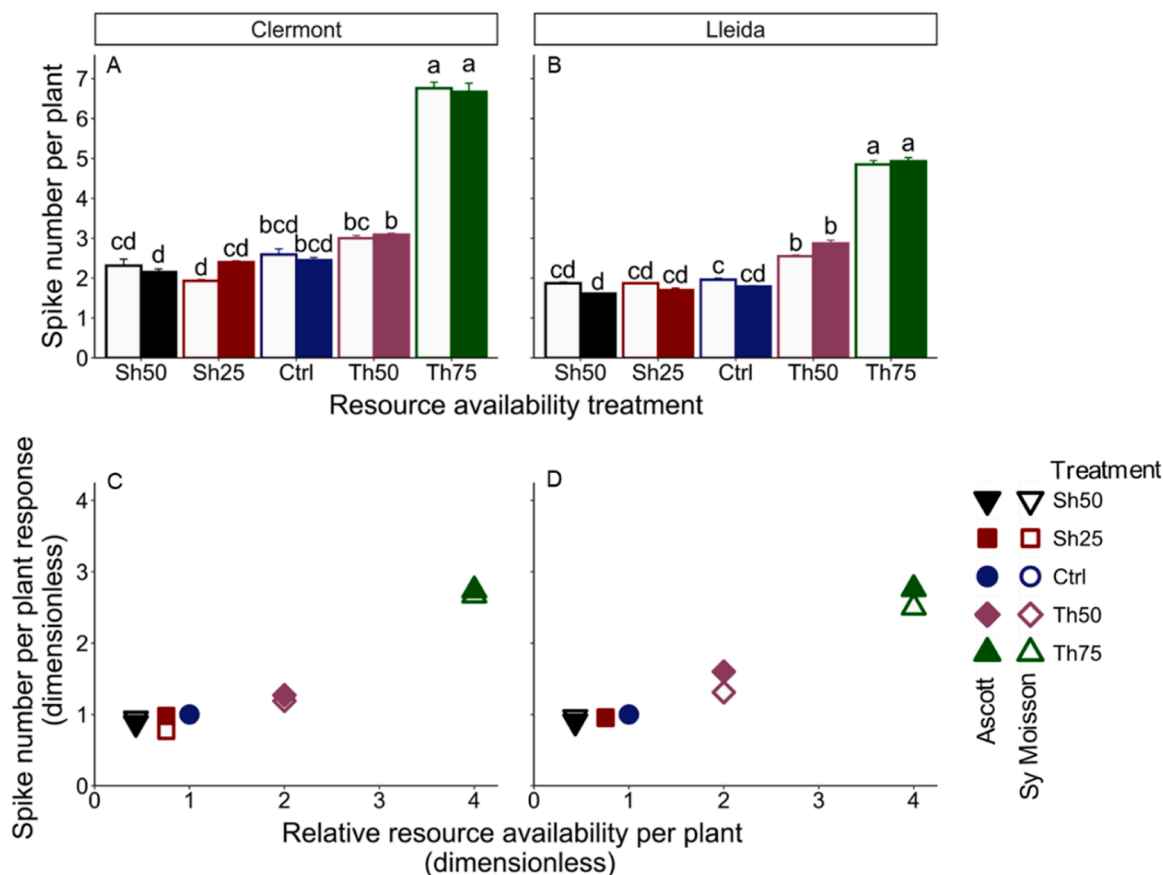
Fig. 6. The upper panels represent the spike number per plant of Ascott (solid bars) and Sy Moisson (open bars) for each treatment in Clermont (A) and Lleida (B). The error bars represent the standard error and different letters represent significant difference according to ANOVA's LSD for each location. The lower panels represent the relationship between spike number per plant response (i.e., the treatment value relative to the control within each genotype) and resource availability for each genotype-treatment in Clermont (C) and Lleida (D). Resource availability is expressed in relative terms to the control, which was fixed as 1, on each location as represented in the scale of Fig. 2. Symbols as in Fig. 3.

underlines why the "critical period" for grain number (and yield) determination is the phase immediately before flowering; as revealed by the pioneer work of Tony Fischer (1985) and confirmed, time and time again, by a plethora of following studies in wheat (see many references in Slafer et al., 2023a and Fischer et al., 2024) and in field crops in general (Carrera et al., 2024). Grain number reductions and increases in response to shading and thinning treatments were less than proportional. For instance, averaging across locations and cultivars, Sh50 reduced grain number by approximately 24.8 %, even though radiation was reduced by 50 %. Similarly, Th75 increased grain number by roughly threefold when resource availability was assumed to increase fourfold. This lack of proportionality between the treatment magnitudes and grain number responses suggests that the assumed change in growth resources was overestimated. Specifically, (i) shading might have increased the proportion of diffuse, thereby enhancing the radiation use efficiency of the canopy (Sinclair et al., 1992; Kim et al., 2021), and (ii) thinning would have reduced radiation interception proportionally less than the increase in land area per plant, as the leaf area index during stem elongation under high-yielding conditions is much higher than 1. Indeed, the fact that losses in grain number (and yield) can be substantially less than the magnitude of shading is in line with what has been reported many times (e.g., McMaster et al., 1987; Fischer, 1985; Savin and Slafer, 1991; Abbate et al., 1995; 1997; González et al., 2005a, 2005b; Mu et al., 2010; Li et al., 2010), although there were also cases in which the response to shading (e.g., Slafer et al., 1994) or thinning (Fischer and Laing, 1976) were similar to the potential change in availability of resources.

4.1. Plasticity of GN and its components SN and GS

We found direct evidence confirming that grain number was more plastic in Ascott than in Sy Moisson. This difference in plasticity was due to their differential plasticity for the two main components of grain number, SN and GS, although they seemed not equally consistent, as the higher plasticity of SN in Ascott was statistically significant only in one of the two locations, whilst the higher plasticity of GS was consistently observed in both Clermont and Lleida. Thus, even though SN proved to be very plastic as a trait (as discussed more in detail below), the difference in GN plasticity between Ascott and Sy Moisson was mostly due to their differences in GS plasticity.

We imposed the treatments at the onset of stem elongation when tillering is expected to be mostly finished (Slafer et al., 2021). Tillering is known to be very plastic (Sadras and Rebetzke, 2013), but the tillering seems not to be a critical period for grain number determination (Reynolds et al., 2022; Slafer et al., 2023b). Thus, our treatments could only affect SN through affecting the survival of tillers produced before stem elongation. Even when the treatments were imposed after the maximum number of tillers had been produced, they affected dramatically SN (evidencing that tiller survival is as critical as, if not more critical than, tillering for the determination of SN). Indeed, SN plasticity (for both cultivars and in both locations) was not only very high but also much higher than GS plasticity (SN increased by 2.5- to 3-fold in response to Th75, when GS increased between 1- to 1.5-fold). This supports the hypothesis that SN would act as a coarse-tuning component when the changes in availability of resources is large and GS would be a relatively fine-tuning mechanism. This had been hypothesized in the
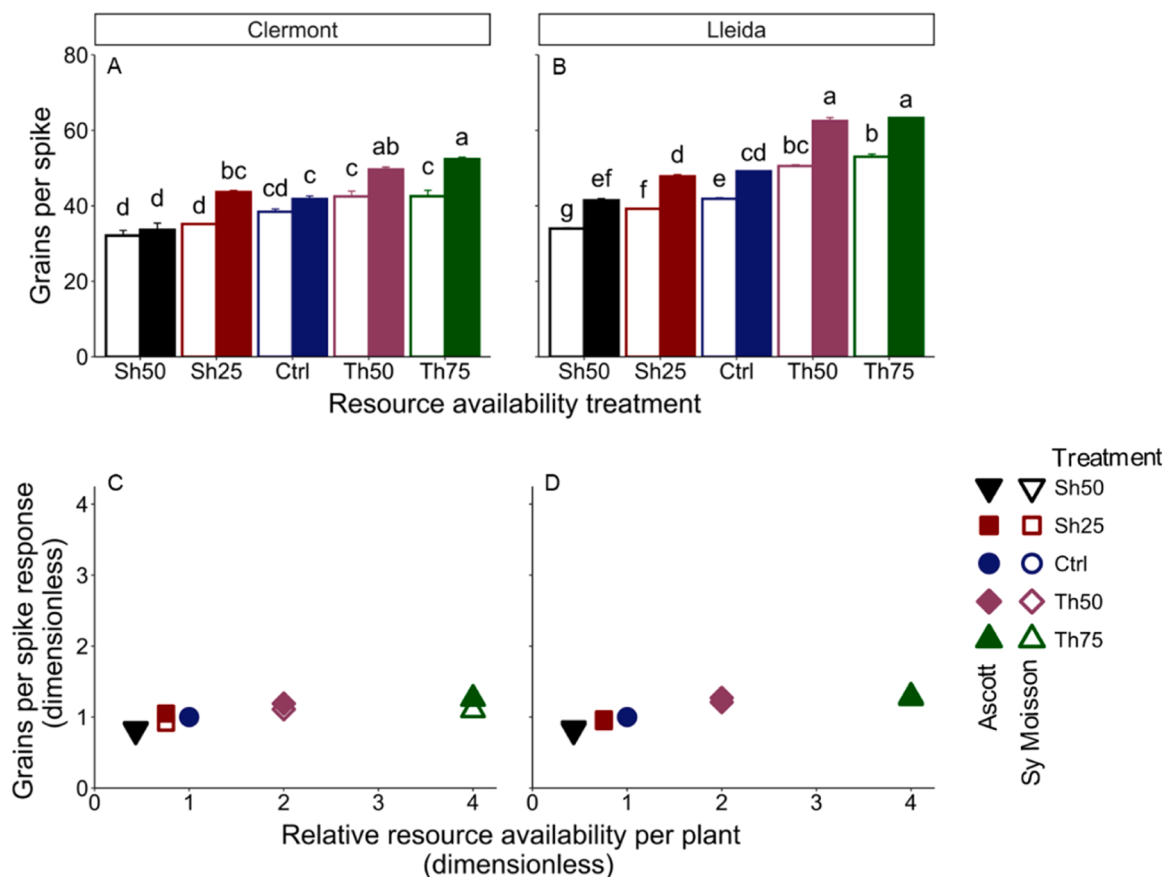
Fig. 7. The upper panels represent the grains per spike of Ascott (solid bars) and Sy Moisson (open bars) for each treatment in Clermont (A) and Lleida (B). The error bars represent the standard error and different letters represent significant difference according to ANOVA's LSD for each location. The lower panels represent the relationship between grains per spike response (i.e., the treatment-genotype value relative to its control – fixed in 1) and resource availability for each genotype-treatment in Clermont (C) and Lleida (D). Resource availability is expressed in relative terms to the control which was fixed as 1 on each location as represented in the scale of Fig. 2. Symbols as in Fig. 3.
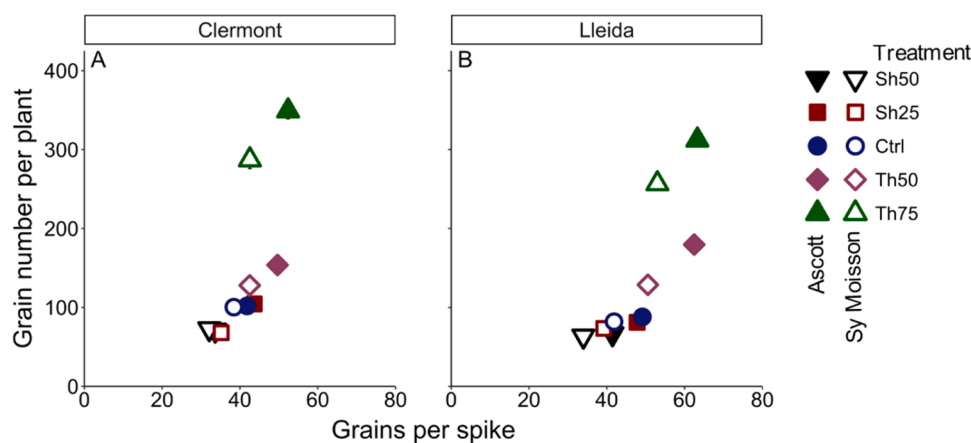

Fig. 8. Relationship between grain number and grains per spike for each genotype-treatment in Clermont (A) and Lleida (B). Symbols as in Fig. 3.

past from analyses of published data (Sadras and Slafer, 2012; Slafer et al., 2022, 2014) but not directly determined in experiments designed explicitly for determining the responses of yield determinants to manipulations imposing a large range of conditions, as in the present study. It may seem noticeable that while SN responded very strongly to increases in resource availability (thinning the crop) it was virtually unresponsive to reductions in resources (shading). Indeed, should this experiment be conducted only with shading treatments the conclusion would have been that SN is very conservative. Most likely, this was the

consequence of having used a sowing density that in the conditions of our experiments resulted in a relatively low number of spikes per plant. That means that tiller mortality would have been already significant in the controls. If plants in the control had only 2.5 or 2 spikes per plant (Clermont and Lleida, respectively), it seems almost impossible that a treatment restricting the availability of resources during stem elongation could reduce SN further. In other words, when considering the responsiveness of a trait defining the structure of the canopy (as tillering defining the number of shoots) we must be aware that such plasticity
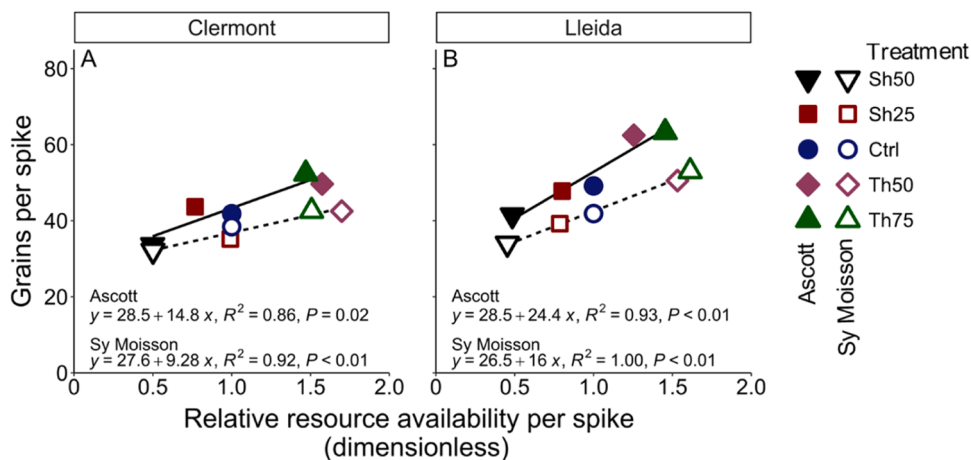
Fig. 9. Relationship between the number of grains per spike and the relative resources per spike for each genotype-treatment in Clermont (A) and Lleida (B) along with the linear regression parameters (R^2 and slope $\pm$ standard error) for Ascott (solid lines) and Sy Moisson (dashed lines). Considering 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 x-axis is dimensionless and represents the resource availability per spike according to the relative spike population m^{-2} a treatment had compared to its control for each location. That is, a higher value means less spikes m^{-2} for the same availability of resources. After considering spike population variations, reductions in incoming radiation were directly discounted for shading treatments. Symbols as in Fig. 3.
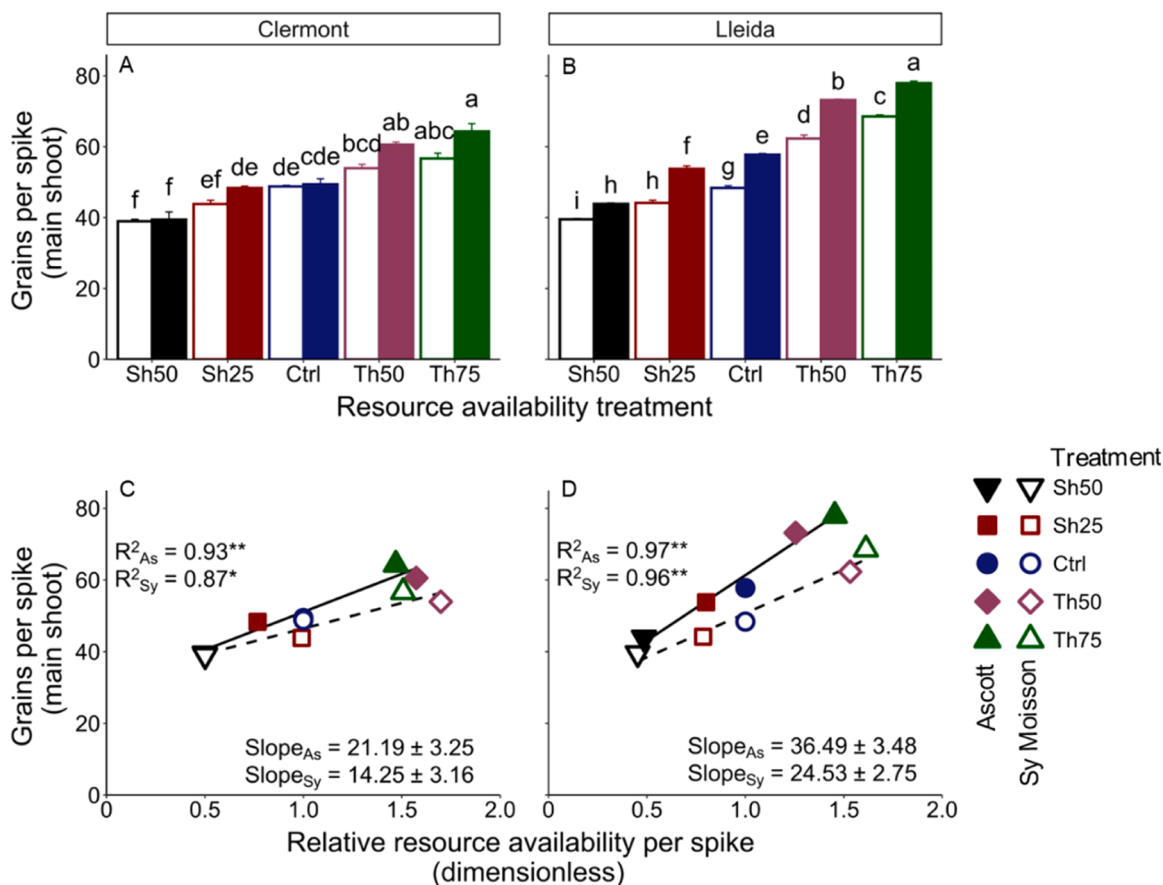

Fig. 10. The upper panels represent the grains per spike in main shoot of Ascott (solid bars) and Sy Moisson (open bars) for each treatment in Clermont (A) and Lleida (B). The error bars represent the standard error and different letters represent significant difference according to ANOVA's LSD for each location. The lower panels represent the relationship between the number of grains per spike and the relative resources per spike for each genotype-treatment in Clermont (C) and Lleida (D) along with the linear regression parameters (R^2 and slope $\pm$ standard error) for Ascott (As, solid lines) and Sy Moisson (Sy, dashed lines). 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 x-axis is dimensionless and represents the resource availability per spike according to the relative spike population m^{-2} a treatment had compared to its control for each location. That is, a higher value means less spikes m^{-2} for the same availability of resources. After considering spike population variations, reductions in incoming radiation were directly discounted for shading treatments. Symbols as in Fig. 3.
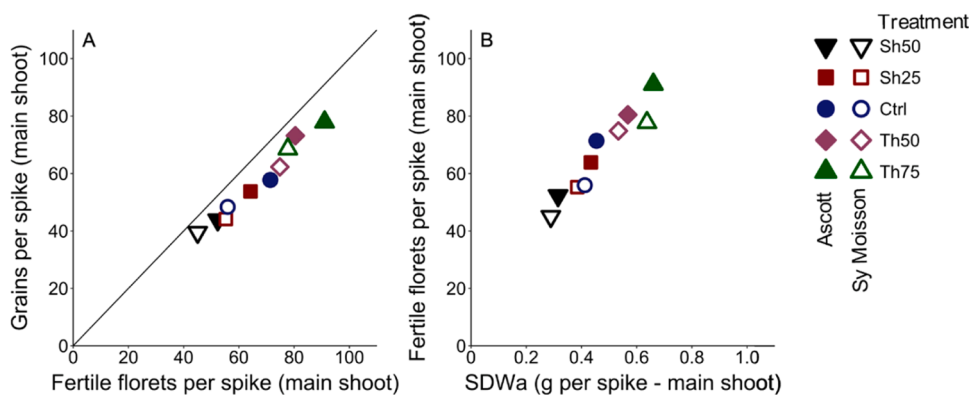
Fig. 11. Relationships between (i) the number of grains (at maturity) and fertile florets (at anthesis) per spike (A), and (ii) the number of fertile florets per spike and spike dry weight at anthesis (SDWa) for each genotype-treatment in Lleida considering only the main shoot spikes where fertile florets were determined. Symbols as in Fig. 3. Line in panel A is the 1–1 ratio.

would not be independent of the structure in the control. We would speculate that in a different canopy structure, with plants producing more fertile spikes in the control (e.g., at a much lower plant density), SN would have been as plastic to reductions in resources as it was shown to be responsive to improvements in resources here. Indeed, this had been discussed, though not directly determined, that the effect of shading on SN would be positively related to the number of spikes per plant in the unshaded control (i.e., if the control had many spikes per plant shading would reduce SN while if the number of spikes per plant is low shading would not affect it; see discussion in Slafer et al., 1994). If the canopy structure implies more than 3 spikes per plant, shading during stem elongation does reduce SN noticeably (Slafer et al., 1994), while when the canopy structure determines that SN is made of main-shoot spikes with the contribution of the first tiller spike at the most, shading during stem elongation does not (cannot) affect SN (Savin and Slafer, 1991).

Unlike with SN, GS was affected in both directions by the treatments: it was decreased when the availability of resources per spike was reduced and *vice-versa*. The shading treatment could not kill a well-developed tiller, however the available resources in these treatments were not enough to produce spikes as fertile as those of the control, causing a reduction of GS in both Sh25 and Sh50. On the other hand, with the thinning treatment not only the spikes of the main shoot were more fertile than those of the control, but also the spikes from tillers contributed to maintaining a higher average GS. By increasing the proportion of spikes coming from tillers in the thinned treatments increasing SN, it might be expected an increase in relatively smaller spikes originated from latest tillers that contribute with less grains and so diluting the GS of the crop. The elevated resource availability in both thinning treatments was sufficient to allow the survival of many tillers still maintaining a reasonably high fertility preventing a trade-off between GS and SN.

The higher GS of Ascott may be, at least partially, explained by its constitutive higher number of spikelets per spike compared to Sy Moisson. The treatments, however, were applied at the onset of stem elongation which is later than terminal spikelet setting. That is, when treatments were imposed, the crop had the number of spikelets per spike established and from then onwards, the spike fertility was altered through changes in the number of grains within the already fixed number of spikelets according to the availability of resources resulting from the treatments. Ascott had more grains per spike than Sy Moisson within each of the treatments, indicating a putative higher GS. But more interesting than the intrinsic higher fertility of Ascott, is its response to the different levels of resource availability. The GS plasticity in Ascott was higher when analysing its relationship with the relative available resources per spike. Not only a steeper reduction with shading was observed but also a higher increase with thinning. The latter may offer

perspective to use existing genetic variability for the capacity to compensate for SN decrease by increased GS. In the case of a putative early cycle stress leading to SN reduction and the associated increase in available resource level per spike, our results suggest that an Ascott-type genotype would exhibit a more stable GN than a type of genotype like Sy Moisson.

4.2. Responses of AGW

It may have been expected that the increase in availability of resources per plant (and per spike) during grain filling due to the thinning treatments would have increased AGW. However, far from this we observed either no change (Clermont) or even a decrease (Lleida) in AGW of the thinned plots compared to the control. This may well be the consequence of the large increase in grain number due to thinning: when grain number rise noticeably the proportion of constitutively smaller grains increase, which leads to a reduction in AGW (Miralles and Slafer, 1995; Acreche and Slafer, 2006) even in the absence of significant competition among grains during grain filling (Slafer et al., 2021). Thus, this also means that the availability of resources during the effective period of grain filling was rather irrelevant to determine the plasticity of yield. This remarks that:

(i) grain growth is sink-limited (and therefore determined by the potential size of the grains rather than by the availability of resources during grain filling; Borrás et al., 2004; Serrago et al., 2013; Slafer et al., 2023a). and therefore

(ii) crop growth responses to pre-anthesis resources are mechanistically critical to determine yield (Dreccer et al., 2022; Reynolds et al., 2022; Slafer, 2003; Slafer et al., 2023b; Fischer et al., 2024), as it is during this period when post-anthesis sink-strength (number and the potential size of the grains) is being determined (Calderini et al., 2021; Fischer, 2011; Reynolds et al., 2022).

Wheat, as a cleistogamous plant, has a high rate of grain set (i.e., most fertile florets at anthesis become grains at maturity; Slafer et al., 2021), unless a severe stress occur at, or immediately after, anthesis. Indeed, in the present study, we observed that GS was closely related to FF. And the effect of treatments on the plasticity of GS mimicked that on FF, which responded to the variations of resource availability. This is in line with the evidence in the literature indicating that floret development is source-limited and therefore increasing the availability of resources increase the likelihood of labile floret primordia to become FF (Dreccer et al., 2014; Ferrante et al., 2010, 2020; Ghiglione et al., 2008; Gonzalez et al., 2005a, 2005b), as analyzed in detail in the companion paper (Bicego et al., 2024).

5. Conclusions

SN was more plastic than GS when exposed to increases in resource availability (the shading treatments could hardly affect SN if this variable was already very low in the controls). The higher plasticity of SN is in line with expectations from theory (e.g., Sadras and Slafer, 2012) but that had not been evident from meta-analyses combining many studies together (Slafer et al., 2014).

Based on multilocal field trials we hypothesized that there was strong differences between genotypes for GS plasticity. When subjected to manipulations in growing conditions imposed directly as treatments, the behaviour of the two extreme genotypes selected, provide evidence for our hypothesis. This hypothesis on genotypic variation in plasticity of GS derived from multilocation and multiyear datasets, and the consistency of the genotypic difference across the two locations, suggest that the genotypic difference in plasticity is rather constitutive and would therefore be useful in designing strategic crosses aiming to combine plasticity of GS with pother yield-related attributes.

The plasticity of GS was tightly related to that of FF and therefore studying the dynamics of floret development and allocation of resources to the juvenile spikes is relevant to understand better the mechanisms determining the differential plasticity of GS between genotypes.

CRediT authorship contribution statement

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

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.fcr.2024.109653.

Data availability

Data will be made available on request.

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