



Grain yield and kernel setting of multiple-shoot and/or multiple-ear maize hybrids

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ABSTRACT

Rainfed maize (*Zea mays* L.) crops in low-rainfall regions are cultivated at very low plant population densities that favor the production of fertile tillers or multiple ears in the main shoot. Little information exists regarding the functional processes governing grain yield and kernel setting on ears of different order of shoots of maize crops. To study these functional processes, field experiments were conducted cultivating two commercial maize hybrids (AX7784 and DM2738) under supplementary irrigation using different plant densities, N rates at sowing, shading around female flowering (silking) and tiller removal treatments. Hybrids differed in the number of tillers per plant at R₁ (AX7784 > DM2738). Early tiller removal increased main shoot growth rate around silking, suggesting competition for light among shoots. Kernel number per plant was positively and curvilinearly related with plant growth rate around silking, i.e. critical period (PGR_{CP}), in tillered and non-tillered plants of both hybrids. Tillered plants set higher kernel numbers than non-tillered plants at PGR_{CP} > 9 g pl⁻¹ d⁻¹ (AX7784) or 11 g pl⁻¹ d⁻¹ (DM2738), while the opposite trend occurred at PGR_{CP} values < 9 g pl⁻¹ d⁻¹ (both hybrids). In both hybrids, the combination of a higher biomass partitioning to but a lower reproductive efficiency of ears of tillers than of main shoots resulted in a lower kernel number for tillers than for main shoots. Genotypic differences were evident in kernel setting between main shoots (DM2738 > AX7784) and tillers (AX7784 > DM2738). The different pattern of kernel setting between hybrids was explained by differences in the biomass partitioning to tiller ears and the reproductive efficiency of ears of tillers (AX7784 > DM2738), and both the biomass partitioning to and the reproductive efficiency of second and third order ears of main shoots (DM2738 > AX7784). Kernel number per plant was higher for DM2738 due to a higher kernel setting on multiple ears of main shoot plus tillers (more prolific and with lower tiller fertility) than for AX7784 (less prolific and with higher tiller fertility). However, grain yield did not differ between hybrids, because of the higher kernel weight of AX7784. Therefore, this work opens avenues for exploiting higher grain yields in maize crops with low plant population densities through the combination of prolificacy and tillering. Further studies should analyze the impact of reproductive plasticity on kernel weight determination in low-density maize crops.

Abbreviations: EGR_{CP}, ear growth rate during the critical period; MSE1, first order ear of the main shoot; MSE2, second order ear of the main shoot; MSE3, third order ear of the main shoot; MSE1GR_{CP}, growth rate of the first order ear of the main shoot during the critical period; MSE2GR_{CP}, growth rate of the second order ear of the main shoot during the critical period; MSE3GR_{CP}, growth rate of the third order ear of the main shoot during the critical period; MSEGR_{CP}, growth rate of any ear of the main shoot during the critical period; MSGR_{CP}, main shoot growth rate during the critical period; PGR_{CP}, plant growth rate during the critical period; SGR_{CP}, shoot growth rate during the critical period; TE1, first order ear of a tiller; TE1GR_{CP}, growth rate of the first order ear of a tiller during the critical period; TE2, second order ear of a tiller; TE2GR_{CP}, growth rate of the second order ear of a tiller during the critical period; TGR_{CP}, tiller growth rate during the critical period; Vn, n ligulated leaf stage.

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1. Introduction

Rainfed maize (*Zea mays* L.) crops in temperate semi-arid regions with high inter-annual variation of summer rainfall, are commonly cultivated at very low plant population densities (i.e., less than 4 plants m^{-2}). Examples of these regions are the western Corn Belt of the United States (Grassini et al., 2015), the Southwestern Pampas of Argentina (Rotili et al., 2019), and Northern New South Wales, the Darling Downs and Central Queensland in Australia (Rodríguez et al., 2017; Rotili et al., 2020). At very low plant densities, maize hybrids may produce fertile tillers (shoots of secondary order which provide vegetative and reproductive plasticity) (Nielsen, 2003; Thomison, 2017; Rotili et al., 2021a, 2021b) and/or set kernels on first order (MSE1) and second order ears (MSE2) of the main shoot (reproductive plasticity only) (Ciancio et al., 2016). Both are reproductive strategies that contribute to grain yield at low plant densities (Ross et al., 2020; Veenstra et al., 2021) by increasing ears and kernel number per unit area. However, little information exists regarding the functional processes governing kernel setting in shoots and ears of different orders (Rotili et al., 2021b).

Considering non-prolific maize hybrids (one ear on a single-shoot plant), kernel number per plant relates asymptotically with plant growth rate during the critical period (PGR_{CP}) (Andrade et al., 1999), while for prolific maize hybrids (more than one ear on a single-shoot plant), kernel number per plant features a more linear response to PGR_{CP} due to the contribution of kernels of MSE2 at high PGR_{CP} values (Ciancio et al., 2016). For the non-prolific hybrids, the function relating kernel number per plant and PGR_{CP} has three parameters with biological meaning: i) the minimum threshold PGR_{CP} for kernel set, below which the plant is sterile; ii) the curvilinearity of the response of kernel number per plant to increases in PGR_{CP} above the minimum PGR_{CP} threshold, i.e., kernel set efficiency at low PGR_{CP} values and iii) the maximum number of kernels for MSE1, given by morphogenetic limitations. Recently, the kernel number per plant- PGR_{CP} function has been comparatively parameterized for tillered and non-tillered plants of the same maize hybrid (Rotili et al., 2021a). In that study, the tillered plants set lower kernel numbers per plant than non-tillered plants at low values of PGR_{CP} (lower than ca. $7 \text{ g pl}^{-1} \text{ d}^{-1}$), but greater kernel numbers per plant at PGR_{CP} higher than ca. $7 \text{ g pl}^{-1} \text{ d}^{-1}$ promoted by kernel setting in ears of tillers. Additionally, tillered plants reached higher PGR_{CP} values than the non-tillered plants. Despite the originality of the data presented by Rotili et al. (2021a), some limitations of the analysis should be noted to identify the knowledge gaps relating to the phenomena involved. For example, sterile plants were not recorded, hence the minimum threshold PGR_{CP} for kernel set in the tillered and non-tillered plants was extrapolated from the fitted function. Moreover, the relationship between kernel number per plant and PGR_{CP} in tillered plants should be understood through the sum of independent kernel set functions for shoots of different order. This analysis should involve the relationships between (i) kernel number of the main shoot as a function of main shoot growth rate during the critical period ($MSGR_{CP}$) and (ii) kernel number of tillers as a function of tiller growth rate during the critical period (TGR_{CP}) (Rotili et al., 2021b). This would also allow a comparison of the functions fitted to each shoot order.

Another important issue that requires consideration is that Rotili et al. (2021a) did not delve into the underlying mechanisms of the kernel number per plant vs PGR_{CP} relationship (Andrade et al., 1999; Vega et al., 2001). Particularly, for multiple-shoot crops, these mechanisms would involve the partitioning of biomass to the ears in the shoots of different order around the critical period (as reflected in the relationship between ear growth rate of tillers or main shoot and shoot growth rate during the critical period (EGR_{CP} and SGR_{CP} , respectively) and the reproductive efficiency of the ears of shoots of different order (as reflected in the relationship between kernel number per ear and EGR_{CP}). Based on this physiological framework, prolific hybrids, had a higher PGR_{CP} threshold for biomass partitioning to MSE2 than to MSE1 and a lower reproductive efficiency of MSE2 than MSE1 (Ciancio et al., 2016),

suggesting that lower-order structures of the main shoot such as third-level ears (MSE3) would also feature those characteristics. It would be of interest to compare all mentioned traits in maize hybrids with different mechanisms of reproductive plasticity, such as multiple shoots per plant or multiple ears per main shoot.

We hypothesized that (i) maize hybrids prone to set more kernels on multiple ears of the main shoot plus on ears of tillers attain higher kernel number per plant and grain yield than maize hybrids prone to set less kernels on multiple ears of the main shoot and with multiple fertile shoots per plant; (ii) in maize hybrids prone to tillering, tillered plants have higher reproductive plasticity than non-tillered plants at high PGR_{CP} values but less reproductive plasticity at low PGR_{CP} values; and (iii) in maize hybrids prone to tillering, tillers are less efficient at setting kernels, i.e. lower kernel number per shoot, than the main shoot due to a lower biomass partitioning to and/or a lower reproductive efficiency of ears of tillers than those of the main shoot. The objective of this work was to understand the physiological determinants of grain yield and kernel setting in ears of different order of main shoot and tillers, i.e. those traits involved in the reproductive plasticity of low-density maize crops. To test the hypotheses and achieve the objective two commercial maize hybrids differing in the numbers of fertile tillers and of ears on main shoot (Rotili et al., 2021b) were cultivated under supplementary irrigation at different plant densities, with different N supply, and with shading during the critical period and tiller removal treatments.

2. Materials and methods

2.1. Crop management

Field experiments were carried out in the experimental unit of the Department of Plant Production, Universidad de Buenos Aires, Argentina ($34^{\circ} 35' \text{ S}$, $58^{\circ} 29' \text{ W}$) on a deep silty clay loam soil (Vertic Argiudoll; Soil Science Division Staff, 2017) during three consecutive growing seasons (Experiment 1: 2017–2018; Experiment 2: 2018–2019; Experiment 3: 2019–2020). Two semi-dent commercial maize hybrids, previously classified (Rotili et al., 2021b) by their reproductive plasticity (AX7784VT3PRO from the company Nidera Semillas, with restricted prolificacy and prone to tillering, and DM2738MGRR from the company Don Mario Semillas, prone to present multiple ears per main shoot and with restricted tillering; AX7784 and DM2738 as from here, respectively) were cultivated. Both hybrids are tolerant to glyphosate [N-(phosphonomethyl) glycine] and widely used by farmers in Argentina. Experiment 1 and Experiment 2 were partially described only for AX7784 in Rotili et al. (2021a). Weeds were chemically controlled using atrazine (2.5 kg ha^{-1}), acetochlor (3.5 l ha^{-1}) and halosulfuron-methyl (120 g ha^{-1}) before crop emergence, and glyphosate (2.5 l ha^{-1}) after crop emergence. Insect injury was managed chemically using flubendiamide (80 ml ha^{-1}). No disease incidence was registered in any of the three experiments, but intense rainfall events occurred during the initial vegetative stages of Experiment 2 and caused growth restriction during several days due to flooding. Crops were conducted with drip irrigation to ensure adequate water availability throughout the phenological cycle. Air temperature and incident solar radiation were measured with a meteorological station located in the experimental field (Davis, Weather Monitor II).

2.2. Experimental design

2.2.1. Experiment 1 and Experiment 2

Experiment 1 was sown on November 17, 2017, and Experiment 2 was sown on November 6, 2018. A split-plot randomized complete block design with three replicates was used, with N treatment (N-: 60 kg N ha^{-1} and N+: 220 kg N ha^{-1}) in the main plot and the combination of tiller removal, plant density and hybrid in the sub-plot (hereinafter termed plot). Initial soil N availability was determined prior to sowing (N-) and N fertilizer (N+) was added as urea after sowing. Hybrids were

cultivated at two plant densities (2 and 4 pl m⁻²) in plots of eight (2 pl m⁻²) or six (4 pl m⁻²) rows 10 m long 0.5 m apart. To ensure the stand of plants, three seeds per hill were manually sown and later thinned by hand to one plant per site at the one-ligulated-leaf stage (V₁; Ritchie et al., 1993). An additional plot cultivated at 2 pl m⁻² was included in each block in which tillers were removed daily between their emergence (ca. V₅) and the end of the tiller emission phase (ca. V₁₀). Every tiller was carefully removed by hand taking care not to damage the main shoot. This treatment was included to ensure that non-tillered plants were sufficiently represented at low plant density and to compare the functional relationships of kernel setting of tillered and non-tillered plants of the same hybrid.

2.2.2. Experiment 3

Experiment 3 was carried out with the aims of i) characterizing the number of tillers per plant at R₁ under a wide range of plant densities and ii) explore the kernel setting functions of different plant categories (tillered and non-tillered), shoot categories (main shoots and tillers) and ear categories (MSE1, MSE2, MSE3, TE1 – first order ear of the tiller –, TE2 – second order ear of the tiller –) for a wider range of growth rates during the critical period than those explored in Experiments 1 and 2. For each hybrid, treatments involved: 16 pl m⁻², 8 pl m⁻², 4 pl m⁻² and 2 pl m⁻² without shade, and 4 pl m⁻² and 2 pl m⁻² shaded. Shading (65% reduction of incident solar radiation) was achieved by installing black nets 0.4 m above the top of the canopy from V₁₃ to R₂₋₃ stages. As tillers usually appear and grow at low plant densities, shading was only applied to the 2 and 4 pl m⁻² plots. This shading treatment was included to promote low growth rates around the critical period for tillered plants that usually have higher growth rates than non-tillered plants (Rotili et al., 2021a).

Hybrids were sown on December 17, 2019, in rows 0.5 m apart. The individual plant was considered as the sample unit, so plants were cultivated in plots in the field and ten to thirty plants (depending on the density) were afterwards randomly selected within the central rows of each plot to perform measurements. Plots involved four rows 2 m long (16 pl m⁻² and 8 pl m⁻²), four rows 4 m long (4 pl m⁻²) or eight rows 6 m long (2 pl m⁻²). For 2 pl m⁻² and 4 pl m⁻², half of the plot area was assigned to shading treatments. All plots were irrigated and fertilized with 200 kg N ha⁻¹ and tillers were never removed.

2.3. Measurements

2.3.1. Phenology and growth of vegetative and reproductive organs

Ten to thirty plants with similar phenological stage were tagged at V₃ in the central rows of each plot (the number of tagged plants varying between experiments and densities). For each tagged plant, phenology was followed on main shoots and tillers, when tillers were present (Experiments 1, 2 and 3). Daily observations of silk extrusion (i.e., at least one silk observed outside the husks) in every ear of main shoot and tillers of tagged plants were performed daily, beginning at the date when the first plant of each plot started silk extrusion (Experiments 1, 2 and 3). For each tagged plant, the number of tillers at R₁ was registered.

In all experiments, non-destructive allometric measurements (Rotili et al., 2021a) were performed on the tagged plants to estimate vegetative biomass at the following stages: (i) ca. 15 days before silking of the first order ear of the main shoot (ca. V₁₃ stage), (ii) silking of the first order ear of the main shoot, (iii) silking of the first order ear of tillers, (iv) 15 days after silking of the first order ear of the main shoot and (vi) 15 days after silking of the first order ear of tillers. On each main shoot or tiller, measurements involved maximum and minimum stem base diameter and height from the ground level to the insertion of the last expanded leaf. Allometric models were also used to estimate biomass of the first, second and third order ears of the main shoot and tillers of each tagged plant at silking date of each ear and 15 days after that date using measurements of the maximum diameter of the ears. Measurements of stem or ear diameter were taken with an electronic digital vernier

caliper while the measurements of shoot height were taken with a tape measure.

In Experiments 1 and 2, non-tagged plants in the central rows of each plot were sampled throughout the crop cycle (until R₂), including the ontogenetic stages in which tagged plants were measured (for AX7784 totals of: 855 main shoots, 558 main shoot ears, 710 tillers and 323 tiller ears; for DM2738 totals of: 862 main shoots, 688 main shoot ears, 284 tillers and 203 tiller ears). Non-destructive allometric measurements described above were performed on the main shoot, tillers and ears, when they were present, of these sampled plants. Immediately after measurements, plants were separated in main shoot and tillers, and the vegetative (stalk + leaves + tassel) and reproductive (husks + cob + florets) biomass of each shoot category (main shoot and tillers) was identified. Biomass was oven dried at 65 °C until constant weight. Allometric relationships were established between the morphometric variables and the dry weights of main shoots, tillers and ears. Vegetative biomass (stalk + leaves + tassel) of main shoots or tillers was estimated based on a quadratic function (Eq. (1)) fitted to the shoot dry weight and stem volume relationship (Table S1) using the cylinder volume equation (Vol; cm³).

$$\text{Vegetative biomass(g)} = a\text{Vol(cm}^3) + b\text{Vol(cm}^3)^2 \quad (1)$$

where a is the parameter of the linear term, b is the parameter of the quadratic term and Vol is the cylinder volume (cm³) based on mean stem diameter and stem height (cm³). The function was forced through zero so that it was biologically meaningful.

The biomass of each ear (husks + cob + florets) of main shoot and tillers at silking date and 15 days after that date was estimated with an exponential function (Eq. (2)) fitted to ear dry weight and maximum ear diameter (mm) relationship of sampled ears of main shoots and tillers (Table S1):

$$\text{Ear biomass(g)} = c^{\text{Diam (mm)}} \quad (2)$$

where c and d are the constants of the exponential function, and Diam is the ear diameter (mm).

The total biomass of each shoot in each ontogenetic stage resulted from the sum of the vegetative and reproductive biomass (the biomass of every measured ear if present). This non-destructive technique has been widely used in maize to measure the growth of tagged plants remaining in the field until harvest, in both non-prolific and prolific maize genotypes (Andrade et al., 1999; Vega et al., 2001; Echarte et al., 2004; Rossini et al., 2011; Laserna et al., 2012; Ciancio et al., 2016) and recently in tillered and non-tillered plants (Rotili et al., 2021a).

For each tagged plant, MSGR_{CP}, TGR_{CP} (distinguishing each individual tiller), MSEGR_{CP} and TEGR_{CP} (distinguishing each ear if present) were estimated for the critical period. MSGR_{CP} and TGR_{CP} were estimated as the sum of the growth rates of vegetative and reproductive organs. Growth rates were estimated from the slope of the linear regression fitted to the estimated vegetative biomass or ear biomass at three stages and time from sowing in days (Rossini et al., 2011; Ciancio et al., 2016): – 227 °Cd (ca. 15 days, V₁₃ stage) before silking date of each ear, silking date of each ear and 15 days after silking date of each ear. Thermal time was calculated using 8 °C as the base temperature (Kiniry, 1991). Ear biomass was assumed to be negligible at – 227 °Cd before silking date of each ear (Otegui and Bonhomme, 1998). The growth rate of a tiller that did not reach silking was estimated as the slope of the linear regression fitted to estimated vegetative biomass of that tiller at the pre-silking, silking and post-silking measurement dates of the first order ear of the main shoot of the plant and time in days from sowing. Total TGR_{CP} per plant was calculated as the sum of growth rates of all tillers of the plant. Following the methodology used by Rotili et al. (2021a), PGR_{CP} was estimated as the sum of MSGR_{CP} and total TGR_{CP} of each plant.

2.3.2. Kernel number in different orders of shoots and ears and grain yield components

At physiological maturity, tagged plants were individually harvested and the number of ears per plant (distinguishing between ears of main shoot and ears of tillers) was registered. Kernel number of each ear was counted manually. Total kernel number of tillers per plant was calculated as the sum of kernels of all tillers present in the plant; kernel number per plant was calculated as the sum of kernels of every ear of main shoot and tillers in each plant. Kernel-like structures in the tassels of the tillers (Moullia et al., 1999) were not considered as they are non-marketable due to their exposure to pests and weather conditions because of the lack of husks. Total, main shoot and tiller ears per square meter were calculated as the respective average ear number per plant multiplied by plant density. Kernel number per square meter was calculated as the average kernel number per plant multiplied by plant density. Individual kernel weight (dry weight basis) was estimated weighing 500 kernels per plot using a digital balance. Grain yield per square meter was calculated as kernel number per square meter multiplied by kernel weight.

2.4. Data analysis

2.4.1. Tillers per plant, shoot and ear growth rates, kernel setting of different shoots and ears, grain yield and grain yield components

The datasets of Experiments 1 and 2 were subjected to an analysis of variance (ANOVA) to evaluate the effects of the main treatment factors (N, density and hybrid) and their interactions on the number of tillers per plant at R_1 (not including the tiller removal treatment), ears per square meter, main shoot ears per square meter, tiller ears per square meter, kernel number per square meter, kernel weight, grain yield per square meter, PGR_{CP} , $MSGR_{CP}$, total TGR_{CP} per plant, kernel number per main shoot, total kernel number of tillers per plant and kernel number per plant. Experiment 1 and 2 were analyzed separately fitting a fixed linear model including the effects of N, density and hybrid, considering the split-plot design. P-values of the differences among mean values were considered when comparing the effects of treatments.

The dataset of Experiment 3 was subjected to a multiple-means comparison test using the Bonferroni correction to test the effect of density, hybrid and their interaction on the number of tillers per plant at R_1 , considering the individual plant as the sample unit. The shaded plots were not included in this analysis. The analysis of variance and the multiple-means comparison test were performed using Infostat version 2020 (Di Rienzo et al., 2020).

2.4.2. Functional relationships

Functional relationships describing kernel setting at the plant and shoot level were fitted for each hybrid (Experiments 1, 2 and 3 including shaded and non-shaded plots). Kernel number per plant was related to PGR_{CP} for tillered and non-tillered plants (plants without tiller emission and de-tillered plants) following the equation used by Rotili et al. (2021a) (Eq. (3)). Models for kernel number per shoot vs SGR_{CP} were fitted considering main shoots and tillers as independent categories by using the same model used at the whole plant level.

$$y_1 = e \{1 - \exp[-(x_1 - f)]/g\} \text{ if } x_1 \geq f \quad (3)$$

$$y_1 = 0 \text{ if } x_1 < f$$

where y_1 is kernel number per plant or kernel number per shoot and x_1 is PGR_{CP} or SGR_{CP} . Parameter e quantifies potential kernel number per plant or kernel number per shoot value, parameter g is a measure of the curvilinearity of the relationship. A large g value indicates that the curve approaches a straight line. Parameter f ($g \text{ pl}^{-1} \text{ d}^{-1}$) represents the x_1 threshold value below which kernel number per plant or kernel number per shoot = 0.

Biomass partitioning to the sum of the ears of each shoot order was evaluated using a bi-linear model (Eq. (4)) fitted to the relationship

between total EGR_{CP} vs the corresponding SGR_{CP} (Experiments 1, 2 and 3), following the methodology used by Ciancio et al. (2016) and considering individual main shoots and tillers. This procedure was also followed for biomass partitioning to each ear of each shoot order, considering individually ears from the main shoot and from the tillers.

$$y_2 = h j + i (x_2 - j) \text{ if } x_2 > j \quad (4)$$

$$y_2 = h x_2 \text{ if } x_2 < j$$

where y_2 is the individual (E1, E2, E3 if present) or sum of $MSEGR_{CP}$ or $TEGR_{CP}$ and x_2 is $MSGR_{CP}$ or TGR_{CP} . Parameter h quantifies the initial slope, parameter i quantifies the second slope and parameter j is the breaking-point value of x_2 for the change of slopes.

The reproductive efficiency of each order of ear in each order of shoot was evaluated through fitting a model relating kernel number of $MSE1$, $MSE2$ or $MSE3$ (if present) vs $MSEGR_{CP}$ and kernel number of $TE1$ or $TE2$ vs $TEGR_{CP}$ (Experiments 1, 2 and 3) with Eq. (5) following the methodology used by Echarte et al. (2004).

$$y_3 = [k (x_3 - l)] / [1 + m (x_3 - l)] \text{ if } x_3 \geq l \quad (5)$$

$$y_3 = 0 \text{ if } x_3 < l$$

where y_3 is kernel number of $MSE1$, 2 or 3 or kernel number of $TE1$ or 2 and x_3 is the $MSEGR_{CP}$ or $TEGR_{CP}$ of each individual ear. Parameters k and m represent the initial slope and the curvilinearity of the relationships. Parameter l quantifies the threshold x_3 value for $y_3 > 0$.

Eqs. (3) to (5) were fitted and plotted for each hybrid considering the whole dataset of Experiments 1, 2 and 3 (including shaded and non-shaded plants) using GraphPad Prism (GraphPad Prism version 6.00 for Windows, 2012). Comparisons of fitted models between the corresponding plant categories (tillered and non-tillered), shoot categories (tillers and main shoots), sum of ears between shoot categories and ear categories ($MSE1$, $MSE2$, $MSE3$, $TE1$ and $TE2$) were performed using the extra sum-of-squares F test ($\alpha = 0.05$) in the model comparison tool in GraphPad Prism. Model comparison was performed testing the null hypothesis: a curve for the different plant, shoot or ear categories in each hybrid (referred as a "Global" curve). The alternative hypothesis of the test was: a different curve for the different plant, shoot or ear categories in each hybrid.

2.4.3. Comparisons of kernel setting for different plant, shoot and ear categories at different plant, shoot and ear growth intervals

There is a possibility of some dissimilarities in the distributions of the residuals for the relationships described in Eqs. (3) and (5) at different interval values of the independent variable. Thus, the comparison between the fitted models could hinder the adequate discrimination between the behavior of different categories (plants, shoots or ears) for certain intervals of values of the independent variable. An alternative and complementary approach to test these different patterns is the one used by Echarte et al. (2004) and Cerrudo et al. (2020) to compare kernel number per plant between hybrids at different PGR_{CP} intervals. Following this methodology, an analysis was performed to the whole data sets of Experiments 1, 2 and 3 (including shaded plants) to compare kernel numbers between categories of plants (tillered vs non-tillered plants), shoots (main shoots vs tillers) and ears (different orders of ears) at discrete intervals of the total range of values for the independent variables (PGR_{CP} , $MSGR_{CP}$, TGR_{CP} , EGR_{CP}). Comparisons were performed between: (i) different categories within the same hybrid and (ii) the same category between hybrids. Non-tillered plants included plants without tiller emission and de-tillered plants. Main shoots of tillered and non-tillered plants were grouped together in the same category. For each hybrid, intervals for PGR_{CP} of $2 \text{ g pl}^{-1} \text{ d}^{-1}$ (with exception for a first interval of $3.89 \text{ g pl}^{-1} \text{ d}^{-1}$ due to non-explored lower PGR_{CP} values by tillered plants and a second interval ranging from 3.90 to $4.99 \text{ g pl}^{-1} \text{ d}^{-1}$ to assure sufficiently large sample sizes across intervals), intervals for both $MSGR_{CP}$ and TGR_{CP} of $1 \text{ g shoot}^{-1} \text{ d}^{-1}$ or for EGR_{CP} of $1 \text{ g ear}^{-1} \text{ d}^{-1}$

were determined and *t* tests were used to assess differences on kernel number in each interval of the independent variable.

3. Results

3.1. Environmental conditions

During the tiller emission period (V_5 - V_{10} , data not shown), accumulated incident photosynthetically active radiation was slightly higher (Experiment 1 = 374 MJ m⁻², Experiment 2 = 391 MJ m⁻², Experiment 3 = 378 MJ m⁻²) and mean air temperature slightly lower (Experiment 1 = 25.4 °C, Experiment 2 = 22.8 °C, Experiment 3 = 25.2 °C) in

Experiment 2 than in the other two experiments. Considering the whole crop cycle and the 30-day period bracketing R_1 , accumulated incident photosynthetically active radiation and mean air temperature also differed between Experiments (Experiment 1 = 2468 MJ m⁻² and 722 MJ m⁻²; 24.2 °C and 25.2 °C; Experiment 2 = 2585 MJ m⁻² and 637 MJ m⁻²; 22.7 °C and 24.1 °C; Experiment 3 = 2499 MJ m⁻² and 711 MJ m⁻²; 22.1 °C and 23.7 °C).

3.2. Expression of multiple-shoot and multiple-ear phenotypes

Tiller number per plant differed among experiments. Particularly, in Experiment 2, flooding at early vegetative stages greatly attenuated

Table 1

Mean values of the number of tillers per plant at R_1 , ears per square meter, main shoot ears per square meter and tiller ears per square meter for maize hybrids AX7784 and DM2738 cultivated under two N supplies (low: N- and high: N+) at two plant densities (2 and 4 pl m⁻²) in Experiment 1 and Experiment 2 and mean values of the number of tillers per plant at R_1 without N restrictions at four plant densities (2, 4, 8 and 16 pl m⁻²) in Experiment 3. Data from the tiller removal treatment (Experiments 1 and 2) or shading treatment (Experiment 3) were not included in the analysis of the number of tillers per plant at R_1 and tiller ears m⁻². The results of the analysis of variance (Experiments 1 and 2) and of the multiple comparison of means (Experiment 3) are presented as the *p*-values of the differences between treatments. Values between brackets show the standard error of the mean.

	N	Density pl m ⁻²	Hybrid	Tillers per plant at R_1	Ears m ⁻²	Main shoot ears m ⁻²	Tiller ears m ⁻²	
Exp. 1	N-	2 (-T)	AX7784	-	4.06 (0.06)	4.06 (0.06)	-	
			DM2738	-	5.56 (0.06)	5.56 (0.06)	-	
		2	AX7784	1.83 (0.05)	5.72 (0.34)	2.78 (0.06)	2.94 (0.34)	
			DM2738	1.42 (0.24)	5.94 (0.56)	3.89 (0.86)	2.06 (0.31)	
			AX7784	1.42 (0.05)	6.67 (0.84)	4.89 (0.29)	1.78 (0.62)	
			DM2738	0.11 (0.11)	8.33 (0.33)	7.89 (0.11)	0.44 (0.29)	
	N+	2 (-T)	AX7784	-	3.78 (0.22)	3.78 (0.22)	-	
			DM2738	-	5.67 (0.10)	5.67 (0.10)	-	
		2	AX7784	1.95 (0.12)	5.28 (0.34)	2.39 (0.06)	2.89 (0.34)	
			DM2738	1.31 (0.07)	6.44 (0.22)	4.50 (0.10)	1.94 (0.24)	
	4	AX7784	1.22 (0.17)	6.56 (0.11)	4.44 (0.29)	2.11 (0.40)		
		DM2738	0.47 (0.15)	9.11 (0.11)	8.11 (0.11)	1.00 (0.00)		
	Exp. 2	N-	2 (-T)	AX7784	-	3.94 (0.06)	3.94 (0.06)	-
				DM2738	-	4.33 (0.10)	4.33 (0.10)	-
				2	AX7784	0.31 (0.19)	4.00 (0.50)	3.44 (0.29)
DM2738					0 (0)	4.39 (0.11)	4.39 (0.11)	0
4				AX7784	0.61 (0.36)	4.89 (0.11)	4.56 (0.11)	0.33 (0.19)
				DM2738	0 (0)	6.56 (0.44)	6.56 (0.44)	0
N+			2 (-T)	AX7784	-	4.00 (0.00)	4.00 (0.00)	-
				DM2738	-	5.11 (0.11)	5.11 (0.11)	-
			2	AX7784	0.86 (0.12)	5.44 (0.06)	3.72 (0.28)	1.72 (0.31)
				DM2738	0 (0)	4.56 (0.28)	4.56 (0.28)	0
4	AX7784		0.86 (0.22)	6.89 (0.22)	4.78 (0.29)	2.11 (0.48)		
	DM2738		0 (0)	8.00 (0.00)	8.00 (0.00)	0		
Exp. 3	N		2	AX7784	2.25 (0.09)	p = 0.0034	p = 0.0558	p = 0.0262
				DM2738	2.04 (0.09)	p < 0.0001	p < 0.0001	p = 0.0107
				AX7784	1.83 (0.13)	p = 0.0005	p < 0.0001	p < 0.0001
		DM2738		0.58 (0.13)	p = 0.0827	p = 0.0069	p = 0.0107	
		AX7784		0.4 (0.14)	p = 0.0849	p = 0.0319	p = 0.0009	
		DM2738		0 (0.14)	p = 0.2611	p < 0.0001	p = 0.0013	
		AX7784		0 (0.08)	p = 0.2611	p = 0.0022	p = 0.0180	
		DM2738		0 (0.08)	p = 0.1351			
		Density		p = 0.6320				
		Hybrid		p = 0.0005				
N x Density		p = 0.0827						
N x Hybrid		p = 0.0849						
Density x Hybrid		p = 0.2611						
N x Density x Hybrid		p = 0.2611						
Exp. 3		N		2	Density	p < 0.0001		
	Hybrid		p < 0.0001					
	Density x Hybrid		p < 0.0001					

Table 2

Mean values of crop grain yield per square meter, kernel number per square meter and kernel weight for maize hybrids AX7784 and DM2738 cultivated under two N supplies (low: N- and high: N+) at two plant densities (2 and 4 pl m⁻²). Data from Experiment 1 and Experiment 2. The results of the analysis of variance are presented as the p-values of the differences between treatments. Values between brackets show the standard error of the mean.

	N	Density pl m ⁻²	Hybrid	Grain yield g m ⁻²	Kernel number # m ⁻²	Kernel weight mg
Exp. 1	N-	2 (-T)	AX7784	737 (43)	2142 (47)	345.5 (13.3)
			DM2738	705 (12)	2694 (79)	262.9 (9.5)
		2	AX7784	902 (75)	2657 (47)	341.4 (12.9)
			DM2738	840 (33)	2864 (123)	297.5 (20.1)
			AX7784	999 (80)	3017 (231)	334.9 (3.7)
			DM2738	1010 (45)	3963 (85)	255.3 (7.0)
	N+	2 (-T)	AX7784	767 (7)	2123 (53)	363.8 (7.0)
			DM2738	698 (8)	2620 (56)	269.0 (7.7)
		2	AX7784	917 (43)	2619 (106)	354.5 (0.3)
			DM2738	867 (36)	3034 (95)	286.3 (6.1)
			AX7784	1073 (30)	3143 (14)	343.1 (9.7)
			DM2738	1219 (19)	4403 (117)	279.4 (9.6)
	N			p = 0.2171	p = 0.3521	p = 0.0812
	Density			p < 0.0001	p < 0.0001	p = 0.0499
	Hybrid			p = 0.0491	p < 0.0001	p < 0.0001
	N x Density			p = 0.0009	p = 0.0005	p = 0.1623
	N x Hybrid			p = 0.0326	p = 0.0082	p = 0.0468
Density x Hybrid			p = 0.0024	p < 0.0001	p = 0.0140	
N x Density x Hybrid			p = 0.0270	p = 0.0265	p = 0.0512	
Exp. 2	N-	2 (-T)	AX7784	678 (18)	2016 (86)	338.5 (6.1)
			DM2738	594 (14)	2217 (53)	269.6 (9.1)
		2	AX7784	618 (70)	1852 (238)	338.6 (8.2)
			DM2738	577 (7)	2211 (54)	261.6 (3.5)
			AX7784	724 (42)	2389 (160)	304.4 (3.4)
			DM2738	662 (30)	2703 (102)	245.2 (4.2)
	N+	2 (-T)	AX7784	764 (27)	2112 (63)	362.0 (14.5)
			DM2738	692 (13)	2441 (26)	285.0 (7.4)
		2	AX7784	813 (28)	2470 (64)	336.9 (8.8)
			DM2738	668 (7)	2438 (91)	275.2 (10.0)
			AX7784	1035 (20)	3109 (88)	339.0 (13.4)
			DM2738	1101 (35)	4249 (60)	259.4 (11.8)
	N			p = 0.0022	p = 0.0068	p = 0.0117
	Density			p < 0.0001	p < 0.0001	p = 0.0223
	Hybrid			p = 0.0070	p < 0.0001	p < 0.0001
	N x Density			p < 0.0001	p < 0.0001	p = 0.4206
	N x Hybrid			p = 0.1213	p = 0.0407	p = 0.1853
Density x Hybrid			p = 0.0626	p = 0.0072	p = 0.4110	
N x Density x Hybrid			p = 0.0431	p = 0.0066	p = 0.2324	

(AX7784) or canceled (DM2738) tillering. Thus, at 2 pl m⁻² under high N supply, tiller number per plant at R₁ was lower in Experiment 2 (AX7784 = 0.86 tillers plant⁻¹, DM2738 = 0 tillers plant⁻¹) than in Experiment 1 (AX7784 = 1.95 tillers plant⁻¹, DM2738 = 1.31 tillers plant⁻¹) or Experiment 3 (AX7784 = 2.25 tillers plant⁻¹, DM2738 = 2.04 tillers plant⁻¹) (Table 1). Tiller number per plant at R₁ was not affected by N supply (Experiments 1 and 2) and differed between hybrids (AX7784 > DM2738) across densities (Experiment 2 - p = 0.0005 -) or with higher differences between hybrids at higher densities (Experiments 1 - p = 0.0283 - and 3 - p < 0.0001) (Table 1).

In Experiment 1, DM2738 had more ears per square meter than AX7784, especially at 4 pl m⁻² and in the de-tillered 2 pl m⁻² and under N + (p < 0.0808 for the N x density x hybrid interaction and p < 0.0189 for all double interactions; Table 1). In Experiment 2 the differences in ears per square meter between hybrids across the other factors were similar to those in Experiment 1 (p = 0.0141 for N x density x hybrid interaction; Table 1). This was associated with a higher prolificacy (number of ears in the main shoot) for DM2738 than for AX7784, especially at 4 pl m⁻² and/or under N +, but also evident under the other conditions (p = 0.0069 for the N x hybrid interaction and p = 0.0006 for the density x hybrid interaction in Experiment 1, and p = 0.0022 for the N x density x hybrid interaction in Experiment 2; Table 1). De-tillering increased the number of ears of the main shoot per square meter for both hybrids in Experiment 1 and for AX7784 in Experiment 2 (DM2738 did not tiller). Interestingly, at 2 pl m⁻² without tiller removal under N- in Experiment 1 and under N + in Experiment 2, ears per square meter were higher for AX7784 than for DM2738. This

was associated with a higher tiller fertility for AX7784 in both Experiments, conditioned by density (2 pl m⁻² > 4 pl m⁻²), while N availability only conditioned tiller fertility for DM2738 in Experiment 1 and for AX7784 in Experiment 2 (p = 0.0103 in Experiment 1 and p = 0.0180 in Experiment 2 for N x density x hybrid interaction; Table 1). Therefore, the expression of multiple-shoots and multiple-ears evidenced genotypical differences, with AX7784 as a more tillering-prone, less prolific and higher tiller fertility phenotype, and DM2738 as a less tillering-prone, more prolific and lower tiller fertility phenotype.

3.3. Grain yield and grain yield components at the crop level

In Experiment 1, DM2738 attained the highest grain yield at 4 pl m⁻² under N +, but under N- there was no difference between hybrids. When decreasing the density to 2 pl m⁻², grain yield proportionally decreased more for DM2738 than for AX7784 (p = 0.0270 for N x density x hybrid interaction; Table 2). De-tillering decreased grain yield of both hybrids with a similar magnitude order under both N conditions. In Experiment 2, grain yield was also highest for DM2738 at 4 pl m⁻² under N + and similar between hybrids at 4 pl m⁻² under N- while at 2 pl m⁻² (with and without tiller removal) AX7784 attained the highest grain yield (p = 0.0431 for N x density x hybrid interaction; Table 2). Hence, under both N conditions, AX7784 presented the greatest grain yield stability across densities.

As was mentioned for ears per square meter and grain yield, kernel number per square meter was higher for DM2738 than for AX7784, but the differences between hybrids were larger at 4 pl m⁻² than at 2 pl m⁻²,

Table 3

Mean values for plant growth rate (PGR), the growth rate of the main shoot (MSGR) and of tillers (Total TGR) during the critical period (CP), kernel number per plant, kernel number per main shoot, total kernel number of tillers per plant for maize hybrids AX7784 and DM2738 cultivated under two N supplies (low: N- and high: N+), at two plant densities (2 and 4 pl m⁻²) and 2 pl m⁻² with tiller removal (-T). Data from Experiment 1 and Experiment 2. The results of the analysis of variance of the data from each experiment are presented as the p-values of the differences between treatments. Values between brackets show the standard error of the mean.

Exp.	N	Density pl m ⁻²	Hybrid	PGR _{CP}	MSGR _{CP}	Total TGR _{CP}	Kernel number per plant	Kernel number per main shoot	Total kernel number of tillers per plant
				g pl ⁻¹ d ⁻¹	g shoot ⁻¹ d ⁻¹	g d ⁻¹			
Exp. 1	N-	2 (-T)	AX7784	9.29 (0.12)	8.29 (0.12)	–	1071 (39)	1071 (24)	–
			DM2738	8.32 (0.33)	8.32 (0.33)	–	1347 (24)	1347 (39)	–
		2	AX7784	10.40 (0.56)	6.13 (0.03)	4.27 (0.59)	1329 (62)	753 (25)	576 (67)
			DM2738	10.22 (0.33)	7.08 (0.57)	3.14 (0.26)	1432 (56)	1041 (177)	391 (121)
		4	AX7784	6.44 (0.44)	5.19 (0.08)	1.25 (0.48)	754 (58)	650 (46)	104 (13)
			DM2738	5.92 (0.28)	5.77 (0.08)	0.15 (0.30)	991 (21)	978 (14)	13 (8)
	N+	2 (-T)	AX7784	8.97 (0.30)	8.97 (0.30)	–	1061 (27)	1061 (26)	–
			DM2738	9.15 (0.30)	9.16 (0.30)	–	1310 (28)	1310 (28)	–
		2	AX7784	11.34 (0.50)	5.90 (0.30)	5.44 (0.53)	1309 (53)	691 (19)	619 (36)
			DM2738	10.94 (0.82)	7.70 (0.29)	3.24 (0.54)	1517 (48)	1163 (38)	354 (41)
		4	AX7784	6.70 (0.22)	5.23 (0.28)	1.47 (0.21)	786 (4)	637 (37)	148 (40)
			DM2738	6.86 (0.50)	6.12 (0.53)	0.74 (0.20)	1101 (29)	1038 (36)	63 (17)
	N			p = 0.2077	p = 0.3013	p = 0.1359	p = 0.4054	p = 0.7958	p = 0.4102
	Density			p < 0.0001	p < 0.0001	p < 0.0001	p < 0.0001	p < 0.0001	p = 0.0002
Hybrid			p = 0.2521	p < 0.0001	p = 0.0167	p < 0.0001	p < 0.0001	p = 0.0005	
N x Density			p = 0.7119	p < 0.0001	p = 0.0816	p = 0.0086	p = 0.1231	p = 0.7408	
N x Hybrid			p = 0.2633	p = 0.0044	p = 0.1773	p = 0.0061	p = 0.0044	p = 0.0230	
Density x Hybrid			p = 0.5971	p = 0.0014	p = 0.1642	p = 0.0044	p = 0.0102	p = 0.0085	
N x Density x Hybrid			p = 0.6575	p = 0.0213	p = 0.1115	p = 0.0179	p = 0.0130	p = 0.0223	
Exp. 2	N-	2 (-T)	AX7784	6.83 (0.51)	6.83 (0.29)	–	1008 (43)	1008 (43)	–
			DM2738	5.93 (0.29)	5.93 (0.51)	–	1108 (27)	1108 (27)	–
		2	AX7784	8.36 (1.50)	6.1 (0.18)	2.25 (1.35)	926 (119)	821 (75)	105 (54)
			DM2738	6.14 (0.25)	6.14 (0.25)	0 (0)	1105 (27)	1105 (27)	0 (0)
		4	AX7784	5.20 (0.35)	4.69 (0.16)	0.51 (0.32)	597 (40)	581 (35)	17 (9)
			DM2738	4.17 (0.14)	4.17 (0.14)	0 (0)	676 (25)	676 (25)	0 (0)
	N+	2 (-T)	AX7784	7.30 (0.53)	7.30 (0.53)	–	1056 (13)	1056 (13)	–
			DM2738	6.62 (0.16)	6.62 (0.16)	–	1221 (32)	1221 (32)	–
		2	AX7784	12.16 (0.98)	6.32 (0.12)	5.84 (1.07)	1236 (45)	865 (51)	370 (95)
			DM2738	6.64 (0.30)	6.64 (0.30)	0 (0)	1182 (51)	1219 (32)	0 (0)
		4	AX7784	7.19 (0.19)	5.03 (0.25)	2.15 (0.29)	777 (22)	611 (17)	166 (13)
			DM2738	6.05 (0.05)	6.05 (0.19)	0 (0)	1062 (15)	1062 (15)	0 (0)
	N			p = 0.0377	p = 0.0173	p = 0.0665	p = 0.0075	p = 0.0162	p = 0.0179
	Density			p = 0.0006	p = 0.0003	p = 0.0510	p < 0.0001	p < 0.0001	p = 0.3695
Hybrid			p < 0.0001	p = 0.1437	p = 0.0003	p = 0.0009	p < 0.0001	p = 0.0003	
N x Density			p = 0.0980	p = 0.2649	p = 0.0510	p = 0.0003	p = 0.0330	p = 0.3695	
N x Hybrid			p = 0.0197	p = 0.0355	p = 0.0111	p = 0.0737	p = 0.0035	p = 0.0049	
Density x Hybrid			p = 0.0023	p = 0.0336	p = 0.0095	p = 0.0847	p = 0.0137	p = 0.0239	
N x Density x Hybrid			p = 0.0252	p = 0.1304	p = 0.1057	p = 0.0159	p = 0.0268	p = 0.2105	

especially under N + ($p = 0.0265$ in Experiment 1 and $p = 0.0066$ in Experiment 2 for N x density x hybrid interaction; [Table 2](#)). At 2 pl m⁻², de-tillering decreased kernel number per square meter for both hybrids in Experiment 1 and for AX7784 in Experiment 2 (DM2738 did not tiller). Kernel weight was higher for AX7784 (347 mg grain⁻¹ in Experiment 1 and 337 mg grain⁻¹ in Experiment 2) than for DM2738 (275 mg grain⁻¹ in Experiment 1 and 266 mg grain⁻¹ in Experiment 2), but marginally conditioned by other factors ($p = 0.0140$ for density x hybrid interaction and $p = 0.0468$ for N x hybrid interaction in Experiment 1, and $p = 0.0223$ for plant density and $p = 0.0117$ for N condition in Experiment 2; [Table 2](#)). Across treatments and experiments, grain yield was highly correlated with ears per square meter ($r = 0.82$) and kernel number per square meter ($r = 0.85$), but not correlated with kernel weight ($r = 0.10$).

3.4. Physiological determinants of kernel setting at the plant, shoot and ear levels

In Experiment 1 ($p < 0.0001$) and 2 ($p = 0.0006$), PGR_{CP} of both hybrids was affected by plant density (2 pl m⁻² > 4 pl m⁻²) and only in Experiment 2 AX7784 attained the highest PGR_{CP} values at 2 pl m⁻² under both N conditions ($p < 0.0252$ for N x density x hybrid interaction) ([Table 3](#)). De-tillering decreased PGR_{CP} in both hybrids under both N conditions in Experiment 1 ($p = 0.6575$ for N x density x hybrid interaction) but only under N + for AX7784 in Experiment 2

($p = 0.0252$ for N x density x hybrid interaction) ([Table 3](#)).

In Experiment 1, MSGR_{CP} of DM2738 at 2 pl m⁻² was higher than at 4 pl m⁻² and increased with the de-tillering treatment at 2 pl m⁻² under both N conditions (with a higher increase under N +). MSGR_{CP} of AX7784 also increased with the de-tillering treatment (in addition to a higher increase under N +) and N supplies, but was similar across densities ($p = 0.0213$ for N x density x hybrid interaction; [Table 3](#)). During the critical period around R₁ of tillers, values of total TGR_{CP} were higher for AX7784 than for DM2738 ($p = 0.0167$ for hybrid; [Table 3](#)) and at 2 pl m⁻² than at 4 pl m⁻² ($p < 0.0001$ for density; [Table 3](#)), partially due to the higher tiller number per plant ([Table 1](#)). In Experiment 2, MSGR_{CP} of both hybrids was affected by plant density (2 pl m⁻² > 4 pl m⁻²) but with a higher impact for AX7784 than for DM2738 ($p = 0.0336$ for density x hybrid interaction; [Table 3](#)) and high N supply increased MSGR_{CP} of both hybrids, but with a higher order of magnitude for DM2738 than for AX7784 ($p = 0.0355$ for N x hybrid interaction; [Table 3](#)). For AX7784, de-tillering increased MSGR_{CP} under both N levels ($p = 0.0035$ for N x hybrid interaction; [Table 3](#)). Total TGR_{CP} of DM2738 was 0 as it did not tiller and the total TGR_{CP} of AX7784 increased under high N supply ($p = 0.0111$ for N x hybrid interaction) and decreased at 4 pl m⁻² ($p = 0.0095$ for density x hybrid interaction) ([Table 3](#)) due to changes in individual TGR_{CP} rather than in tiller number per plant ([Table 1](#)).

In Experiment 1, DM2738 set more kernel number per plant than AX7784 irrespective of N supply and plant density, and tiller removal

reduced kernel number per plant of AX7784 under both N conditions but only of DM2738 under N + ($p = 0.0179$ for N x density x hybrid interaction; Table 3). In Experiment 2, kernel number per plant of DM2738 was not affected by density under N + but it was reduced under N- ($2 \text{ pl m}^{-2} > 4 \text{ pl m}^{-2}$), while kernel number per plant of AX7784 was maximized at 2 pl m^{-2} with tillers under both N conditions ($p = 0.0159$ for N x density x hybrid interaction; Table 3). Kernel number per plant only differed between hybrids (DM2738 > AX7784) at 2 pl m^{-2} under N-, but under both N supplies kernel number per plant of both hybrids was reduced at 4 pl m^{-2} ($p = 0.0159$ for N x density x hybrid interaction; Table 3).

Tiller removal increased kernel number per main shoot in DM2738 (Experiment 1) and AX7784 (Experiments 1 and 2). Differences in this trait between hybrids were larger under N + and at 4 pl m^{-2} ($p = 0.0130$ in Experiment 1 and $p = 0.0268$ in Experiment 2 for N x density x hybrid interaction; Table 3). Total kernel number of tillers per plant was higher in AX7784 than in DM2738 in Experiment 1 and 2, and the differences were larger under N + at 2 pl m^{-2} ($p = 0.0223$ for N x density x hybrid interaction in Experiment 1 and $p = 0.0049$ for N x hybrid interaction and $p = 0.0239$ for density x hybrid interaction in Experiment 2; Table 3).

In both hybrids, the different kernel number per plant of tillered (total kernel number of tillers per plant + kernel number per main shoot) and non-tillered (only kernel number per main shoot) plants was related to treatment effects on PGR_{CP} ($p < 0.0001$; Fig. 1, top and Table 3 and Table S2). A crossover of the functions fitted to data set of tillered and non-tillered plants arose from values of parameters e and g

(Eq. 3). The model fitted to tillered plants presented a higher maximum kernel number per plant at high PGR_{CP} (parameter e) and a more linear relationship between variables (parameter g), than the model fitted to non-tillered plants. Comparisons of kernel number per plant between plant categories at different ranges of PGR_{CP} confirmed that at $\text{PGR}_{\text{CP}} > 9 \text{ g pl}^{-1} \text{ d}^{-1}$ (for AX7784) or $> 11 \text{ g pl}^{-1} \text{ d}^{-1}$ (for DM2738) tillered plants set more kernel number per plant than non-tillered plants but below $9 \text{ g pl}^{-1} \text{ d}^{-1}$ the opposite occurred in both hybrids (Table 4). Moreover, above $12.8 \text{ g pl}^{-1} \text{ d}^{-1}$ (for AX7784) or $13.4 \text{ g pl}^{-1} \text{ d}^{-1}$ (for DM2738) only tillered plants set kernels (Fig. 1 top). In tillered plants, DM2738 set more kernels per plant (1724) than AX7784 (1544) at $\text{PGR}_{\text{CP}} > 11 \text{ g pl}^{-1} \text{ d}^{-1}$ (Fig. 1 top and Table 4).

When kernel number per tiller and kernel number per main shoot were plotted against TGR_{CP} (i.e., SGR_{CP}) or MSGR_{CP} (i.e., SGR_{CP}), the curvilinear fitted functions adequately described these relationships ($p < 0.0001$; Fig. 1, bottom and Table S3). Tillers had a lower SGR_{CP} threshold for kernel setting and a more curvilinear response of kernel setting to SGR_{CP} than main shoots, particularly for hybrid AX7784 (see parameters f and g in Table S3). Thus, for AX7784 kernel number per tiller was higher than kernel number per main shoot for SGR_{CP} between 2 and $3 \text{ g shoot}^{-1} \text{ d}^{-1}$ ($p = 0.0470$), and lower between 4 and $5 \text{ g shoot}^{-1} \text{ d}^{-1}$ ($p = 0.0153$) and between 6 and $7 \text{ g shoot}^{-1} \text{ d}^{-1}$ ($p = 0.0187$), while for DM2738 kernel number per main shoot was higher than kernel number per tiller between 4 and $6 \text{ g shoot}^{-1} \text{ d}^{-1}$ ($p < 0.0001$) (Table 5). DM2738 set more kernel number per main shoot than AX7784 for MSGR_{CP} between 4 and $11 \text{ g shoot}^{-1} \text{ d}^{-1}$ ($p = 0.0001$ – 0.0003), while AX7784 set more kernel number per tiller than DM2738 for TGR_{CP} between 1 and $2 \text{ g shoot}^{-1} \text{ d}^{-1}$ ($p = 0.0403$) and between 4 and $5 \text{ g shoot}^{-1} \text{ d}^{-1}$ ($p = 0.0118$). Interestingly, in both hybrids some tillers featured sexual dimorphism in their tassels, setting kernels that afterwards were not harvestable (tassel-ear'd tillers; shown always next to the x-axis (kernel number per tiller = 0) in Fig. 1, bottom).

During the critical period of both hybrids, biomass partitioning to ears of main shoots or tillers varied from 0 to near a 0.75:1 proportion (Fig. 2, top). For similar ranges of MSGR_{CP} and TGR_{CP} ($< 6 \text{ g shoot}^{-1} \text{ d}^{-1}$), biomass partitioning was higher for ears of tillers than of main shoots, as evidenced by a lower value of the parameter j of Eq. (4) in the model fitted to the data set of tillers versus the data set of main shoots ($p < 0.0001$ for differences between shoot categories, Table S4). Hybrids differed in the biomass partitioning to ears of main shoots (DM2738 > AX7784) evidenced by a lower value in the parameter j of Eq. (4) in the model fitted to the data set of the main shoots of DM2738 versus the data set of the main shoots of AX7784. For both hybrids, different SGR_{CP} thresholds for biomass partitioning to different ear orders of the same shoot was found, without evident differences between the same ear order of main shoots and tillers within each hybrid (Fig. 2 bottom and Table S5). The SGR_{CP} thresholds were ca. $1 \text{ g shoot}^{-1} \text{ d}^{-1}$ for MSE1 and TE1, 3 (DM2738) or 5 (AX7784) $\text{g shoot}^{-1} \text{ d}^{-1}$ for MSE2 and TE2 and ca. $6 \text{ g shoot}^{-1} \text{ d}^{-1}$ (DM2738 and AX7784) for MSE3.

In both hybrids, and for similar EGR_{CP} values, kernel number of the first order or second order ears of main shoots were higher than those of the ears of same order on tillers reflecting the higher reproductive efficiency of main shoot ears (Fig. 3 and Table 6). Additionally, in both hybrids the dispersion of kernel setting was higher in kernel number of TE1 than in kernel number of MSE1, even featuring sterile TE1 with $\text{TEGR}_{\text{CP}} > 1 \text{ gr ear}^{-1} \text{ d}^{-1}$. Differences between hybrids were found for i) reproductive efficiency of MSE2 (DM2738 > AX7784; see data in Table 6 and parameters k and l in Table S6), ii) TEGR_{CP} threshold for kernel set in TE1 (AX7784 < DM2738; Fig. 3 and parameter m in Table S6), and iii) kernel setting in MSE3 only in DM2738 (Table 6).

4. Discussion

In rainfed low-yielding environments, risk-averse farmers usually cultivate maize at low plant populations (i.e., lower than 4 pl m^{-2} ; Grassini et al., 2015; Rodriguez et al., 2017; Rotili et al., 2019) to

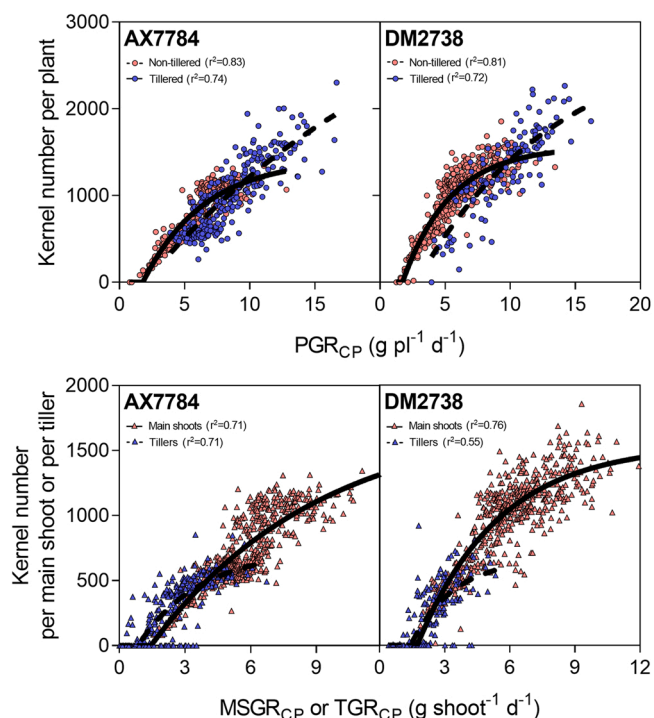


Fig. 1. Top panels: relationship between kernel number per plant and plant growth rate during the critical period (PGR_{CP}) for tillered and non-tillered plants of maize hybrids AX7784 and DM2738. Non-tillered plants included plants without tiller emission and de-tillered plants. Bottom panels: relationship between kernel number per main shoot or kernel number per tiller and main shoot growth rate (MSGR_{CP}) or tiller growth rate (TGR_{CP}) during the critical period for maize hybrids AX7784 and DM2738. Main shoots from tillered and non-tillered plants were considered in the same shoot category for the analysis. Only tillers with $\text{TGR}_{\text{CP}} > 0$ were considered for the analysis. Data from Experiment 1, Experiment 2 and Experiment 3 (including shaded and non-shaded plants). Fitted models were always different when comparing between plant categories (Table S2) or between shoot categories (Table S3).

Table 4

Mean kernel number per plant at different intervals of plant growth rate during the critical period (PGR_{CP}) for tillered (T) and non-tillered (-T) plants of maize hybrids AX7784 and DM2738. Non-tillered plants included plants without tiller emission and de-tillered plants. Number of samples in each plant category x hybrid ranged from 0 to 166 depending on the interval of PGR_{CP}. The lower PGR_{CP} value explored by a tillered plant was 3.91 g pl⁻¹ d⁻¹ for AX7784 and 3.95 g pl⁻¹ d⁻¹ for DM2738. Data from Experiment 1, Experiment 2 and Experiment 3 (including shaded and non-shaded plants).

Hybrid	Tillers	PGR _{CP} intervals (g pl ⁻¹ d ⁻¹)					
		less than 3.89	3.90–4.99	5–6.99	7–8.99	9–10.99	11 or more
		Mean kernel number per plant					
AX7784	-T	325 A	566aB	850aB	1063aB	1094bB	1206bA
	T	–	542 aA	669bA	927bA	1271 aA	1544aB
DM2738	-T	376 A	787 aA	1087 aA	1265 aA	1369 aA	1354bA
	T	–	351bB	808bA	1062bA	1425 aA	1724 aA

- no data.

The two-tailed t test fixed effects model was used to compare means ($p < 0.05$) between plant categories within the same hybrid (low-case letters) and between hybrids within each plant category (capital letters).

Table 5

Mean kernel number per main shoot or per tiller at different intervals of main shoot growth rate (MSGRC_{CP}) or tiller growth rate (TGR_{CP}) during the critical period for maize hybrids AX7784 and DM2738. Main shoots from tillered and non-tillered plants were considered in the same shoot category for the analysis. Number of samples in each shoot category x hybrid ranged from 0 to 149 depending on the intervals of MSGRC_{CP} or TGR_{CP}. Data from Experiment 1, Experiment 2 and Experiment 3 (including shaded and non-shaded plants).

Hybrid	Shoot type	MSGRC _{CP} or TGR _{CP} intervals (g shoot ⁻¹ d ⁻¹)											
		0–1	1–2	2–3	3–4	4–5	5–6	6–7	7–8	8–9	9–10	10–11	11 or more
		Mean kernel number per main shoot or per tiller											
AX7784	Main shoot	0a	101 aA	255bB	464 aA	586aB	671aB	867aB	1031B	1072B	1087B	1111B	1102 A
	Tiller	12 aA	160 aA	332 aA	410 aA	541bA	606 aA	544b	–	–	–	–	–
DM2738	Main Shoot	–	49 aA	344bA	491bA	795 aA	1036 aA	1104 A	1171 A	1311 A	1341 A	1401 A	1354 A
	Tiller	2 A	81aB	265bB	440bA	414bB	622bA	–	–	–	–	–	–

- no data.

The two-tailed t test fixed effects model was used to compare means ($p < 0.05$) between shoot categories within each hybrid (low-case letters) and between hybrids within each shoot category (capital letters).

prevent crop failure in dry years. However, in these environments inter-annual variation of rainfall is usually high (Rotili et al., 2019, 2020, 2021a) and density-independent hybrids are needed to capture unpredictably high resource availability in low-density maize cropping systems (Tokatlidis et al., 2011). Nevertheless, the adequate phenotype to achieve density-independence is not clear. Hybrids should be stress-tolerant to prevent crop failure in restrictive environments (Tollenaar and Lee, 2002), but with a high kernel number per plant to capture better conditions, as both secondary traits of grain yield are independent (González et al., 2018). In this paper we analyzed multiple-shoot and/or multiple ear maize phenotypes. In wet years, commonly used low densities may limit the capture of radiation, biomass and yield, and phenotypes with vegetative and/or reproductive plasticity associated with tillering (Rotili et al., 2021a; b; Veenstra et al., 2021) and/or prolificacy (Ross et al., 2020; Parco et al., 2020) could help to accommodate crop yield to resource surplus through a higher kernel number per plant. In dry years, prolificacy may be canceled (Monneveux et al., 2006) and it has been suggested that tillering could be counter-productive due to excessive vegetative growth that is not translated into reproductive success (Rotili et al., 2021a; b) i.e. a less stress-tolerant pattern. The physiological determinants of kernel setting of multiple-shoot and/or multiple-ear maize hybrids were never compared before and are key to clarify their contribution to crop grain yield in low-density maize crops.

4.1. Under high resource availability, fertile tillers increased grain yield but were not able to compensate a lower number of plants per unit area

Variations of crop grain yield across the explored conditions were positively correlated with the number of ears per square meter and both hybrids exhibited different phenotypical patterns to compose this grain yield component (AX7784 less prolific and with higher tiller fertility than DM2738) (Table 1). In the irrigated experiments of this work, that

resemble environments with high water availability, the high number of ears per plant of the lowest density was not enough to compensate the low number of plants per unit area (2 pl m⁻² versus 4 pl m⁻²), reflected on a lower kernel number m⁻² and crop grain yield (Table 2), as reported previously (Veenstra et al., 2021). Moreover, at 2 pl m⁻², a lower number of tillers per plant (i.e. de-tillering) either decreased (for both hybrids in Experiment 1) or did not modify (for AX7784 in Experiment 2) kernel number m⁻² and grain yield, due to a similar or higher kernel number in the single main shoot, respectively. These results support previous findings that tillers could benefit low-density maize crops in years with unexpected high resource availability (Sangoi et al., 2010; Sangoi et al., 2012a; Rotili et al., 2021a; b; Veenstra et al., 2021), but without fully compensating the expected grain yield m⁻² at a higher density.

Although variations of kernel number m⁻² were positively correlated with crop grain yield, no differences in grain yield between hybrids were detected because the highest kernel number m⁻² of DM2738 (the most prolific hybrid) was counterbalanced by its lowest kernel weight (DM2738 = 249 mg kernel⁻¹; AX7784 = 295 mg kernel⁻¹; across treatments and experiments; Table 2). Based on these results, the first hypothesis (*maize hybrids prone to set more kernels on multiple ears of the main shoot plus on ears of tillers attain higher kernel number per plant and grain yield than maize hybrids prone to set less kernels on multiple ears of the main shoot and with multiple fertile shoots per plant*) is partially rejected. However, the difference in kernel weight between hybrids cannot be directly attributed to the phenotypic pattern, as the determination of kernel weight (Alvarez Prado et al., 2013), tillering or prolificacy (Doebley et al., 1997; Whipple et al., 2011) have different genetic governance. In fact, de-tillering reduced kernel number per plant but had no consistent effect on kernel weight, increasing mean kernel weight for AX7784 but decreasing it for DM2738 (Table 2). Thus, to adequately describe impacts of reproductive plasticity on crop grain yield, further studies should explore the effect of tillering and prolificacy

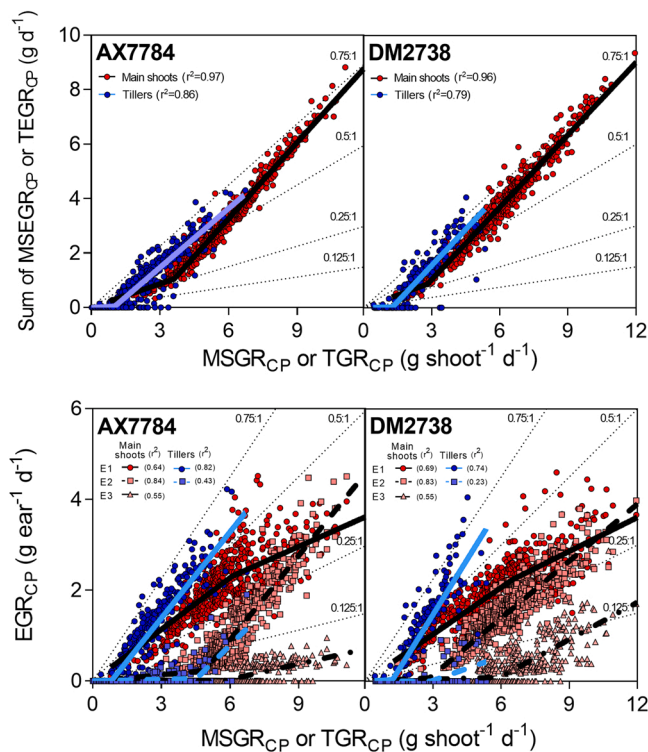


Fig. 2. Top panels: relationship between the sum of the growth rates of ears of main shoot (MSEGR_{CP}) or tillers (TEGR_{CP}) and main shoot (MSGR_{CP}) or tiller growth rates (TGR_{CP}) during the critical period for maize hybrids AX7784 and DM2738; bottom panels: relationship between the growth rate of ears of different order (E1 –first order–, E2 –second order–, E3 –third order–) of main shoot (MSEGR_{CP}) or tillers (TEGR_{CP}) and main shoot (MSGR_{CP}) or tillers growth rate (TGR_{CP}) during the critical period for maize hybrids AX7784 and DM2738. Main shoots from tillered and non-tillered plants were considered in the same shoot category for the analysis. Only tillers with TGR_{CP} > 0 were considered for the analysis. The dotted lines represent different MSEGR_{CP} MSGR_{CP}⁻¹ or TEGR_{CP} TGR_{CP}⁻¹ values. Data from Experiment 1, Experiment 2 and Experiment 3 (including shaded and non-shaded plants). Fitted models were always different when comparing between plant categories in top panels (Table S4) or between shoot categories in bottom panels (Table S5).

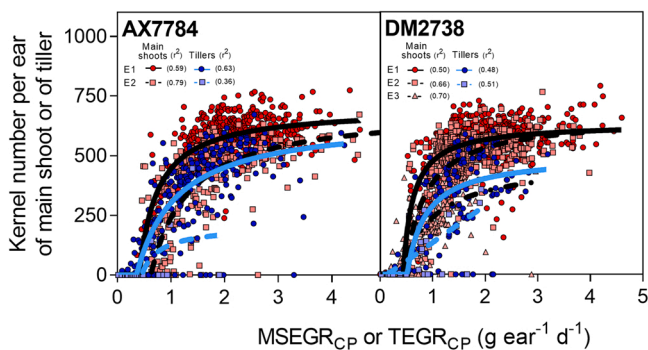


Fig. 3. Relationship between kernel number per ears of different orders (E1 –first order–, E2 –second order–, E3 –third order–) of main shoot or tillers and ear growth rate for the main shoot (MSEGR_{CP}) or the tillers (TEGR_{CP}) during the critical period for maize hybrids AX7784 and DM2738. Main shoots from tillered and non-tillered plants were considered in the same shoot category for the analysis. Data from Experiment 1, Experiment 2 and Experiment 3 (including shaded and non-shaded plants). In AX7784, MSE3 did not set kernels. Fitted models (Table S6) were always different when comparing between ear categories.

Table 6

Mean kernel number at different intervals of ear growth rate during the critical period (EGR_{CP}) for ears of different orders of main shoots or tillers of maize hybrids AX7784 and DM2738. Main shoots from tillered and non-tillered plants were considered in the same shoot category for the analysis. Number of samples in each ear order x shoot category x hybrid ranged from 0 to 251 depending on the interval of EGR_{CP}. Data from Experiment 1, Experiment 2 and Experiment 3 (including shaded and non-shaded plants).

Hybrid	Ear category	EGR _{CP} intervals (g ear ⁻¹ d ⁻¹)				
		0–1	1–2	2–3	3–4	4 or more
Mean kernel number per ear of main shoot or tiller						
AX7784	MSE1	301 aAa	557 aAa	596 aAa	604 aAa	626 aAa
	MSE2	53bAb	377bAb	506bb	522bb	566a
	MSE3	–	–	–	–	–
DM2738	TE1	134aBa	383aBa	502Ba	352B	570 A
	TE2	10bBb	224 aAa	–	–	–
	TE3	–	–	–	–	–

- no data.

X only 1 value.

The two-tailed t test fixed effects model was used to compare means (p < 0.05) between different ear category within each shoot category of the same hybrid (low-case letter), between the same ear category of different shoot categories of the same hybrid (capital letter) and the same ear category of each shoot category between hybrids (letter in italics).

on kernel weight variability through the impact on physiological mechanisms such as source-sink relationships around silking (Gambín et al., 2006) and during the post-flowering period (Borrás et al., 2004) considering both the effects of higher source (tillering) and/or sink (tillering and prolificacy) values.

4.2. For maize hybrids prone to tillering, differences in the function fitted to kernel number versus PGR_{CP} of tillered and non-tillered plants could explain crop grain yield responses to unexpected environmental conditions

Farmers from regions with high interannual variability of water availability face different scenarios when cultivating low-density maize crops: years with high water availability throughout the crop cycle that allow to explore high PGR_{CP} values; years with high water availability during the initial crop stages that promote tillering, but with terminal water stresses that may constrain PGR_{CP} in different degrees depending on the onset of the stress; and years with low water availability during the whole crop cycle, including a seasonal stress during the critical period (Rotili et al., 2020, 2021a). Although in this work we cultivated crops under irrigated conditions, the different treatments (combination of a wide range of plant densities, shading and N rates) allowed us to explore a wide range of PGR_{CP}, like those probably explored throughout the above-mentioned scenarios (Rotili et al., 2021a). The relationship between kernel number per plant and PGR_{CP} has been proposed (Andrade et al., 1999) to understand reproductive success of maize crops based on the conversion of resource capture to kernels at the plant level, independent from the environmental source of PGR_{CP} restriction (Andrade et al., 2002). In our study, this relationship was described by a curvilinear function independently of the presence or absence of tillers, but differences existed in the magnitude of the response between tillered and non-tillered plants of the same hybrid. The crossover response in which tillered plants set more kernels than non-tillered plants at relatively high PGR_{CP} values, but the opposite occurred at relatively low PGR_{CP} values (Fig. 1, top and Table 4), confirmed the findings of Rotili et al. (2021a) for AX7784 but using a larger dataset and extending this response to DM2738, a hybrid with a different phenotypical pattern.

Based on these results, the second hypothesis (*in maize hybrids prone to tillering, tillered plants have higher reproductive plasticity than non-tillered plants at high PGR_{CP} values but less reproductive plasticity at low PGR_{CP} values*) is not rejected. Consequently, tillered plants have not only a higher vegetative plasticity than non-tillered plants (evidenced as differences in the maximum PGR_{CP} values) but also higher reproductive plasticity (evidenced as differences in the maximum kernel number per plant). This functional response of kernel number per plant explains the yield compensation provided by tillers when planting low or ultra-low densities (i.e. 4 and 2 pl m^{-2} , respectively) in years with higher-than-expected resource availability in some environments (Veenstra et al., 2021). However, in years with restricted conditions during the critical period, tillered crops would set less kernels per square meter than non-tillered crops (Rotili et al., 2021a).

4.3. Differences in kernel setting at the shoot level explained the differential response in kernel number per plant of multiple-shoot and/or multiple-ear maize hybrids to unexpected environmental conditions

Exploring genotypical differences in the functions determining kernel setting at the shoot level (main shoots versus tillers) could be of interest to understand the better-suited strategies for maize husbandry in different scenarios. In our work, the determinants of kernel setting at the shoot level (i.e. main shoot or tillers) were studied through two different approaches: first, the competition between tillers and main shoots for radiation capture (through the direct impact of de-tillering); second, the determinants of kernel setting at the shoot level (tillers and main shoots as separate modules). Differences existed in $MSGR_{CP}$ and in kernel number per main shoot of tillered and de-tillered plants of both hybrids (for DM2738 in Experiment 1, and for AX7784 in Experiments 1 and 2, Table 3), evidencing that around flowering tillers and main shoots compete for radiation with implications for kernel setting (Rotili et al., 2021b). DM2738 featured a higher slope than AX7784 in the relationship between kernel number per main shoot and $MSGR_{CP}$, similar to the genotypical differences in the same relationship of hybrids with different prolificacy found by Ciancio et al. (2016). Something similar was evident when considering the tillers, as DM2738 also featured a higher slope for the relationship between kernel number per tiller and TGR_{CP} , which had never been parameterized before. However, the TGR_{CP} threshold for kernel setting in tillers of DM2738 was higher than for AX7784 (Fig. 1 and Table S3), setting less fertile tillers under the lower range of TGR_{CP} values (Table 5). Thus, differences between hybrids for kernel setting on main shoots (DM2738 > AX7784) were larger than those differences on tillers (AX7784 > DM2738). When combining both approaches used to study kernel setting at the shoot level, it is evident that in tillered crops, $MSGR_{CP}$ is reduced and thus a hybrid with higher kernel setting at lower $MSGR_{CP}$ would maintain higher kernel number per main shoot, and tillers would still contribute to kernel number per plant. Therefore, for both hybrids, the vegetative plasticity of tillers was translated into reproductive plasticity through kernel number per tiller, but the greater contribution of kernel number of MSE2 and MSE3 in DM2738 was critical to the expression of a higher kernel number per plant (and per square meter) in this hybrid.

Based on these results, in scenarios with high water availability (high PGR_{CP}) and profusely tillered crops, DM2738 would set more kernels than AX7784 (Fig. 1, top). In scenarios with no tiller emission due to non-promoting conditions for tillering during the initial stages (Rotili et al., 2021b), but with high or intermediate PGR_{CP} values, DM2738 would also set more kernels per unit area, based on its higher prolificacy. By contrast, in scenarios with high tiller emission due to promoting conditions for tillering during the initial stages, but terminal water stresses and thus intermediate-low PGR_{CP} explored by tillered plants, AX7784 would be the one setting more kernels per unit area. However, considering that in many regions the seasonal growing environment is highly unpredictable, the phenotype with higher kernel number per plant in a larger proportion of conditions for both tillered and

non-tillered plants (in this case, the more prolific DM2738) would be desirable.

4.4. Responses of biomass partitioning and the reproductive efficiency of ears of different order of main shoots and tillers described different phenotypical patterns

Although the general framework for low-density maize crops through the combination of different shoots explained kernel setting at the plant and crop level, the physiological determinants that describe the differences of kernel setting of ears of different order on main shoots and tillers would explain the different phenotypical patterns between hybrids. With that aim, we analyzed the following physiological traits: biomass partitioning (i.e., carbon flux) to ears of different order and the reproductive efficiency of these ears (Andrade et al., 1999; Vega et al., 2001), for both main shoot and tillers. Bi-linear rather than linear models were best fitted to describe total biomass partitioning to the ears of main shoots of both hybrids (Fig. 2, top), as was observed for prolific plants (Andrade et al., 1999) and for other Argentinean hybrids (Echarte et al., 2004). However, the more prolific hybrid, DM2738, featured higher biomass partitioning to second and third order ears than AX7784 through a lower $MSGR_{CP}$ threshold value for biomass partitioning to MSE2 and similar threshold but a higher slope of this relationship for MSE3. Therefore, the combination of high source levels (expressed as high $MSGR_{CP}$ values) with high sink numbers (i.e., several fertile ears and high kernel number per main shoot) would have determined high sink strength, which sustained biomass partitioning to the ears of main shoots in both hybrids (up to 0.75:1) (Marcelis, 1996), but with higher values for DM2738. Even though TGR_{CP} was lower than $MSGR_{CP}$, for both hybrids total biomass partitioning to ears of tillers was higher than to ears of main shoots within the SGR_{CP} intervals shared by tillers and main shoots. This was driven by a higher biomass partitioning to TE1 than to MSE1 in both hybrids, without clear differences between hybrids (Fig. 2, bottom). Therefore, differences among non-tillering hybrids in prolificacy expression (ears per plant greater than 1 but lower than 2) that had been previously associated with different threshold values for biomass partitioning to MSE2 (Ciancio et al., 2016), would also be the main difference between tillering-prone hybrids with different phenotypical patterns. Thus, the differences in MSE3 and/or TE1 and TE2 fertility would only marginally be due to different biomass partitioning and most probably due to the other studied physiological trait: the reproductive efficiency of ears.

The study of the reproductive efficiency of the ears of the different type of shoots allowed us to discriminate the reproductive behavior of tillers and main shoots. In both hybrids, kernel number per MSE1 was higher than kernel number per TE1 throughout the explored range of EGR_{CP} (Fig. 3 and Table 6). Probably, differences in the duration of the floret differentiation phase (longer for MSE1 than for TE1) determined the different potential kernel number per MSE1 and kernel number per TE1 as was previously documented for the differences between MSE1 and MSE2 (Otegui, 1997). Also, the dispersion of kernel number per TE1 values at high $TE1GR_{CP}$ values was considerably higher than the dispersion of kernel number per MSE1 values at high $MSE1GR_{CP}$ values (more notably for DM2738), reflecting greater decoupling between kernel setting and ear growth of tillers, also possibly due to a different floret development of ears of different shoot orders and/or flower synchronies (i.e. rates of silk extrusion). Based on the results related to the physiological determinants of kernel setting at the shoot level, the third hypothesis (*in maize hybrids prone to tillering, tillers are less efficient at setting kernels, i.e. lower kernel number per shoot, than the main shoot due to a lower biomass partitioning to and/or a lower reproductive efficiency of ears of tillers than those of the main shoot*) is partially rejected. Reproductive efficiency also differed between hybrids, as indicated by the higher reproductive efficiency of MSE2 of DM2738, another driver of differences in prolificacy within maize hybrids (Ciancio et al., 2016). Moreover, at high $MSGR_{CP}$ values both hybrids exhibited positive biomass

partitioning to MSE3 but only DM2738 set kernels in MSE3. These differences between hybrids found in ears of main shoots were not evident in tillers, as the reproductive efficiency of TE1 and TE2 was only marginally higher for AX7784. Collectively, the results of our work suggest that the genotypical differences in reproductive plasticity (i.e., the tendency to set kernels in several ears of main shoot or tillers) can be adequately described through the same functional bases previously used to describe kernel setting in first order ears of main shoots.

5. Conclusions

This work showed for the first time the underlying mechanisms governing kernel setting of multiple-shoot and multiple-ear maize hybrids. For example, the hybrid AX7784 tillered more profusely, and featured higher TGR_{CP} , higher biomass partitioning to TE1 and higher reproductive efficiency of TE1, but lower biomass partitioning to MSE2 and MSE3 and lower reproductive efficiency of MSE2 than DM2738. Moreover, AX7784 had sterile MSE3 while DM2738 presented fertile MSE3. These differences generated two contrasting phenotypes, AX7784 as a less prolific and more tillering phenotype and DM2738 as a prolific + tillering phenotype. Hence, maize plasticity is a possible avenue to exploit unpredictably high resource availability in particular years in highly interannually variable environments through higher kernel setting. Giving the findings of this work, the preferable phenotype in these environments would be one with the combination of high prolificacy in the main shoot and the emission of fertile tillers. However, to give appropriate agronomic recommendations, future works should explore the effect of prolificacy and tillering on kernel weight determination and the comparison of grain yield of a large set of hybrids with differential reproductive plasticity strategies and tolerance to abiotic stress across several environments varying in resource availability during the crop cycle.

CRediT authorship contribution statement

Diego Hernán Rotili: Conceptualization, Data curation, Investigation, Methodology, Formal analysis, Writing – original draft, Writing – review & editing, Visualization. **L. Gabriela Abeledo:** Conceptualization, Supervision, Writing – review & editing. **Santiago Martínez Larrea:** Data curation, Investigation, Formal analysis. **Gustavo Ángel Maddonni:** Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Methodology, Writing – review & editing.

Declaration of Competing Interest

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

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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.2022.108471.

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