Field Crops Research 142 (2013) 58–67

Contents lists available at SciVerse ScienceDirect

Field Crops Research

journal homepage: www.elsevier.com/locate/fcr

Heat stress in temperate and tropical maize hybrids: A novel approach for assessing sources of kernel loss in field conditions

J.I. Rattalino Edreira[a,b,*], M.E. Otegui[a]

[a] Instituto de Fisiología y Ecología Vinculado a la Agricultura del Consejo Nacional de Investigaciones Científicas y Tecnológicas (IFEVA-CONICET), Facultad de Agronomía, Universidad de Buenos Aires, Av. San Martín 4453, Buenos Aires, Argentina
[b] Facultad de Agronomía, Universidad Nacional de La Pampa, CC 300, RA 6300 Santa Rosa, LP, Argentina

ARTICLE INFO

Article history:
Received 30 May 2012
Received in revised form
12 November 2012
Accepted 12 November 2012

Keywords:
Maize
Zea mays (L)
Temperate and tropical hybrids
Heat stress
Kernel set
Kernel numbers

ABSTRACT

Temperate and tropical maize differ in their tolerance to heat stress but the ecophysiological bases for genotypic differences are poorly understood. Our objectives were (i) to assess the sources of kernel loss, and (ii) to identify the main differences in these traits among genotypes of contrasting genetic background. We used the classic relationships that associate final kernel number per plant (KNP) with plant (PGR_{CP}) and ear (EGR_{CP}) growth rates during the critical period for kernel set and developed an alternative approach based on the combined analysis of these relationships for assessing sources of kernel loss in field conditions. We identified three sources of loss associated with (i) PGR_{CP} reductions (ΔKNP_1), (ii) changes in biomass partitioning to the ear (ΔKNP_2), and (iii) constraints not directly related to assimilate allocation to the ear (ΔKNP_3). A partitioning index was also established ($PI = EGR_{CP}\ PGR_{CP}^{-1}$). Field experiments included three contrasting maize hybrids (Te: temperate; Tr: tropical; TeTr: Te × Tr) grown under two temperature regimes (control and heated) during daytime hours. We tested heating (ca. 33–40 °C at ear level) along two 15-d periods (GS_1: pre-anthesis; GS_2: from silking onwards). Final KNP was severely reduced by heating, and this negative effect was larger (i) when it occurred during silking (−75% for GS_2) than before anthesis (−52% for GS_1), and (ii) for the Te hybrid (−77%) than the TeTr (−69%) and the Tr (−44%) hybrids. The contribution of each source of loss to the decrease in KNP was 47% for ΔKNP_1, 27% for ΔKNP_2, and 32% for ΔKNP_3. Variations in ΔKNP_2 were explained by changes in PI ($r^2 = 0.85$, $P < 0.001$), and a critical PI value (0.25) for avoiding kernel loss due to ΔKNP_2 was established. A similar pattern among genotypes was found for the response of KNP to variations in both PGR_{CP} and EGR_{CP}, but the new approach indicated that enhanced tolerance of the tropical genotype was mainly associated with reduced ΔKNP_3.

1. Introduction

Maize (*Zea mays* L.) grain yield is closely associated with kernel number at harvest, and this yield component depends on the physiological condition of the crop around flowering (Schoper et al., 1982; Kiniry and Ritchie, 1985; Aluko and Fischer, 1988; Grant et al., 1989). Therefore, the variation in kernel number per plant (KNP) has been associated with the variation in plant growth rate during this critical period (PGR_{CP}) under a wide range of environmental conditions (Tollenaar et al., 1992; Andrade et al., 1999, 2002; Vega et al., 2001), including heat stress (Cicchino et al., 2010b). Critical physiological traits that emerge from the analysis of KNP-PGR_{CP} relationship are: (i) the maximum number of kernels set at high availability of resources per plant, (ii) the response of KNP to PGR_{CP} increments, and (iii) the minimum PGR_{CP} threshold for kernel set (Andrade et al., 1999; Echarte et al., 2004; Echarte and Tollenaar, 2006). Nevertheless, ear growth rate during the critical period (EGR_{CP}) is usually a better predictor of KNP than PGR_{CP}, because it eliminates the variation induced by changes in biomass partitioning to the ear (Echarte and Tollenaar, 2006).

The described conceptual framework has been used to identify the physiological traits associated with high tolerance to abiotic stresses, chiefly water deficit (Echarte and Tollenaar, 2006), crowding (Vega et al., 2001; Pagano and Maddonni, 2007), and

Abbreviations: Exp_n, experiment n; EGR_{CP}, ear growth rate during the critical period for kernel set; GS_n, growth stage n; H, hybrid; HE, heat effect; KNP, kernel number per plant; PI, partitioning index; PKNP, potential KNP; $PKNP_{PGR}$, PKNP estimated from PGR_{CP}; $PKNP_{EGR}$, PKNP estimated from EGR_{CP}; PGR_{CP}, plant growth rate during the critical period for kernel set; T_C, non-heated control plot; Te, temperate hybrid; TeTr, Te × Tr hybrid; T_H, heated plot; Tr, tropical hybrid; TR, thermal regime; ΔKNP_1, loss in PKNP due to PGR_{CP} reduction; ΔKNP_2, loss in PKNP due to changes in biomass partitioning to the ear; ΔKNP_3, loss in PKNP due to constraints not directly related to assimilate allocation to the ear.

* Corresponding author at: Instituto de Fisiología y Ecología Vinculado a la Agricultura del Consejo Nacional de Investigaciones Científicas y Tecnológicas (IFEVA-CONICET), Facultad de Agronomía, Universidad de Buenos Aires, Av. San Martín 4453, Buenos Aires, Argentina.
E-mail address: rattalino@agro.uba.ar (J.I. Rattalino Edreira).

0378-4290/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.fcr.2012.11.009

N deficiency (D'Andrea et al., 2008). The physiological bases of heat stress tolerance did not receive much attention until recently (Cicchino et al., 2010b). A better understanding is needed on this topic to address the potential effect of global warming on crops (Parry et al., 1999; Schmidhuber and Tubiello, 2005), especially in high-yielding temperate environments (Monfreda et al., 2008) where substantial crop yield losses are expected due to extreme temperature episodes (Teixeira et al., 2011). The development of genotypes with combined features of high tolerance to heat stress and high yield potential will be critical for these environments.

In a recent research (Rattalino Edreira and Otegui, 2012) on the response of temperate and tropical maize hybrids to brief episodes of above-optimum temperature around flowering, the authors documented a superior performance of the tropical genotype. The advantage of this genetic background seemed related to reduced kernel abortion (Rattalino Edreira et al., 2011) and stable harvest index (Rattalino Edreira and Otegui, 2012) under heat stress, but no link was established between observed differences in grain yield and the response of KNP to assimilates production (e.g., PGR_{CP}) or reproductive growth (e.g., EGR_{CP}).

As for other abiotic stresses (op.cit.), the superior performance of the tropical genotype under heat stress might be attributable, at least in part, to a high ability to sustain plant growth and assimilate partitioning to the ear, a low threshold value of PGR_{CP} for avoiding plant barrenness, and/or a reduced response of KNP to PGR_{CP} variations for minimizing kernel loss when PGR_{CP} declines. Genotypic differences in the response to heat stress, however, could also be attributable to other limiting factors that are not directly related to assimilate availability per plant. These limiting factors are generally associated with severe constraints or failures in reproductive processes, such as reduced pollen shed (Schoper et al., 1987) and pollen viability (Herrero and Johnson, 1980; Mitchell and Petolino, 1988), poor synchrony between anthesis and silking (Cicchino et al., 2010a; Rattalino Edreira et al., 2011), fertilization problems (Dupuis and Dumas, 1990), and/or kernel abortion (Cheikh and Jones, 1994). Because these constraints are usually overexpressed under abiotic stress, they are responsible of the lack of fit in the response of KNP to PGR_{CP} or to EGR_{CP}. In these circumstances, the use of conventional analysis, such as least squares regression, gives a weak prediction of KNP because the estimate develops through the center of data distribution (Cade et al., 1999). This statistical weakness leads to poor estimation of final kernel numbers and may ignore part of the variation in this trait, attributable to the direct effects of heat on kernel set. The latter may be associated with the sensitivity of mentioned reproductive processes, and is expected to be reduced among hybrids with tropical genetic background (Rattalino Edreira et al., 2011). An enhanced interpretation of the variation in KNP to changes in PGR_{CP} under heat stress may be achieved when the analysis is performed near the upper bound (e.g., uppermost 99th quantile) rather than along the center of data distribution. It could be hypothesized that values near this upper boundary represent the potential response of KNP to changes in PGR_{CP}, while the distance between this upper limit and observed KNP is associated with other limiting factors not included in the proposed model. We speculate that heating around flowering may enhance the gap between actual and potential KNP, especially in temperate genotypes.

The objectives of the current research were to (i) assess the causes of kernel loss that account for the gap between actual and potential KNP, and (ii) identify the main differences in these traits among genotypes of contrasting genetic background exposed to contrasting thermal regimes around silking. For addressing the first objective we proposed an alternative approach to the classic curvilinear models fitted independently to the KNP-PGR_{CP} and KNP-EGR_{CP} relationships. This approach is based on the combined analysis of these relationships and the use of the upper bound fit (99th quantile regression). Its application helped us to identify three sources of kernel loss, one related to PGR_{CP} reductions, another one to changes in biomass partitioning to the ear, and a third one not directly related to assimilate allocation to the ear (e.g. pollination failure, lack of ovary fertilization and/or kernel abortion).

2. Materials and methods

2.1. Crop husbandry and treatment description

Field experiments were conducted during 2008–2009 (Exp_1) and 2009–2010 (Exp_2) at the experimental field of the University of Buenos Aires, Argentina (34°35′S, 58°29′W) on a silty clay loam soil (Vertic Argiudoll; USDA soil survey system). Treatments included a factorial combination of (i) three F1 hybrids (H) of contrasting genetic background (Te: temperate, Tr: tropical, and TeTr: temperate × tropical), (ii) two temperature regimes (T_C: control with no heating, T_H: heated) applied during daytime hours (ca. 33–40 °C at ear level), and (iii) three different growth stages (GS). Only two stages were included in the current analysis, those that covered the first (GS_1: 15 days before anthesis) and the second (GS_2: 15 days from start of silking onwards) half of the critical period for kernel set (ca. 30 d around silking; Fischer and Palmer, 1984; Kiniry and Ritchie, 1985; Andrade et al., 1999). Hybrids were 2M545 HX (Te), 2B710 HX (Tr), and 2A120 HX (TeTr), all currently produced by Dow Agroscience Argentina for different regions of this country (Rattalino Edreira et al., 2011). In both experiments, a single stand density of 9 plants m^{-2} was used. Crops were fertilized with urea at a rate of 200 kg N ha^{-1} at V_6 (Ritchie and Hanway, 1982). Water availability of the uppermost 1 m of the soil profile was kept near field capacity throughout the growing season by means of drip irrigation. Weeds and insects were adequately controlled. More details about crop husbandry can be found in Rattalino Edreira et al. (2011).

Treatments were distributed in a split split-plot design, with growth stages, hybrids and thermal regimes (TR) in the main plot, subplot and sub-subplot (hereafter termed plots), respectively. Three replicates were always used. Main plots were 10 m length, with six rows separated at 0.5 m between rows. Temperature regimes covered an area of 6 m^2 along the four central rows of each main plot. These areas were enclosed with polyethylene film (100 μm thickness) mounted on wood structures (Cicchino et al., 2010a). For T_C shelters, the lateral films were open up to 1.4 m above soil surface. This was done to avoid differences in light offer due to the polyethylene film. For T_H shelters, the film reached the soil surface on all sides, except one side that had a 10 cm opening at the bottom. Additionally, roofs of all shelters were pierced for avoiding excessive heating at the top of the canopy and for helping with adequate gas exchange. Heating of T_H treatments depended mainly on temperature rise promoted by the greenhouse effect of the polyethylene enclosure (Cicchino et al., 2010a). Nonetheless, shelters for the T_H condition were supplemented with an electric fan heater monitored by an automated control unit (Cavadevices, Buenos Aires, Argentina).

Heating of GS_1 started when 50% of the plants in control plots of each hybrid reached ca. V_{15}-V_{17} (Ritchie and Hanway, 1982), and finished when 10% of these plants reached anthesis. Heating of GS_2 started when 10% of plants in control plots reached R_1 and finished 15 days later. All shelters were removed at the end of each heating period. Different sowing dates were used for each GS × H combination in order to start all heating treatments almost at a same calendar date. This was done to achieve similar stress intensities for avoiding the confounded effect of the environment on treatments evaluation. Additionally, delayed sowing dates (from 2-December

onwards for Exp_1 and from 20-November onwards for Exp_2) were selected for starting the temperature treatments after the period of highest irradiance and temperature, which takes place between late December and the first half of January (Otegui et al., 1996). This was done to avoid over-heating of heated plots. More details about the heating system and heat stress characteristics can be found in Rattalino Edreira et al. (2011).

2.2. Measurements and computations

Nine plants per plot were tagged at V_{11} in both experiments. The ontogeny stages of V_{15}, R_1 and R_2 were registered on these plants, and their shoot biomass at these stages was estimated by means of allometric models based on the relationship between plant biomass and morphometric variables (Vega et al., 2000; Maddonni and Otegui, 2004; Pagano et al., 2007). For all treatment combinations, 12–15 plants of variable size (i.e. plant height, stalk diameter) were harvested at mentioned stages to obtain model parameters. Morphometric measurements included stem diameter at the base of the stalk, plant height from ground level to the collar of the last fully expanded leaf, and maximum ear diameter (only at R_1 and R_2). Fitted models to the relationship between plant biomass and morphometric variables were always significant ($P < 0.001$) and coefficients of determination averaged 0.77 across all treatment combinations. Plant and ear biomass estimated for each tagged plant were used to calculate plant (PGR_{CP}; in g d^{-1}) and ear (EGR_{CP}; in g d^{-1}) growth rates during the critical period for kernel set. These traits were computed as the slope of the linear regression fitted to estimated biomass at V_{15} (only for PGR_{CP}), R_1 and R_2. Ear biomass was assumed to be zero at V_{15} (ca. −227 °Cd before silking; Otegui and Bonhomme, 1998). Biomass partitioning to the ear (PI: partitioning index) was computed for each tagged plant as the quotient between EGR_{CP} and PGR_{CP}. Estimated values of PGR_{CP}, EGR_{CP} and PI were averaged for each plot.

All tagged plants were harvested when 50% of the grains from the mid portion of the ears showed black layer formation (Daynard and Duncan, 1969). The apical ear of each tagged plant was hand shelled for counting the final kernel number per plant (KNP). No subapical ears were detected.

2.3. Statistical analysis

Least squares regression was used to analyze the mean response of actual KNP to variations in PGR_{CP} or in EGR_{CP}. These relationships were fitted to each genotype data set across all treatment combinations by means of the curvilinear model in Eq. (1)

$$\mathrm{KNP} = a_{\mathrm{mean}} \left\{ 1 - \exp \left[\frac{-(X - c_{\mathrm{mean}})}{b_{\mathrm{mean}}} \right] \right\} \quad (1)$$

where X represents either PGR_{CP} or EGR_{CP}, parameter a_{mean} (plateau of the model) is the maximum number of kernels set in the topmost ear, b_{mean} is a measure of the response of KNP to the variation in PGR_{CP} or in EGR_{CP}, and c_{mean} represents threshold PGR_{CP} or EGR_{CP} values for kernel set. Data were analyzed using the *nls* package of R software (R Development Core Team, 2011).

Additionally, we analyzed the response of potential KNP (PKNP) to variations in PGR_{CP} or in EGR_{CP} by means of the 99th quantile regression [Eq. (2)].

$$\mathrm{PKNP} = a_{\mathrm{pot}} \left\{ 1 - \exp \left[\frac{-(X - c_{\mathrm{pot}})}{b_{\mathrm{pot}}} \right] \right\} \quad (2)$$

where X represents either PGR_{CP} or EGR_{CP}, parameter a_{pot} is the absolute potential kernel number per plant, b_{pot} is a measure of the potential response of KNP to PGR_{CP} or EGR_{CP} increments, and c_{pot} represents the minimum PGR_{CP} or EGR_{CP} for kernel set (i.e. threshold value). Data were analyzed using the *quantreg* package of R software (R Development Core Team, 2011), and coefficients of determination of quantile regression analysis (r^1) were computed in terms of weighted sum of absolute residuals (Koenker and Machado, 1999). These values are a measure of the local goodness of fit at a specific quantile and should not be interpreted like the ordinary coefficient of determination of least square regression analysis (i.e. r^2), which measures global goodness of fit. This approach enhances the statistical strength of comparisons (described next) with respect to simple frontier analysis usually performed in resource use efficiency studies (French and Schultz, 1984; Otegui and Bonhomme, 1998). A theoretical representation of models fitted by Eqs. (1) and (2) is shown in Fig. 1.

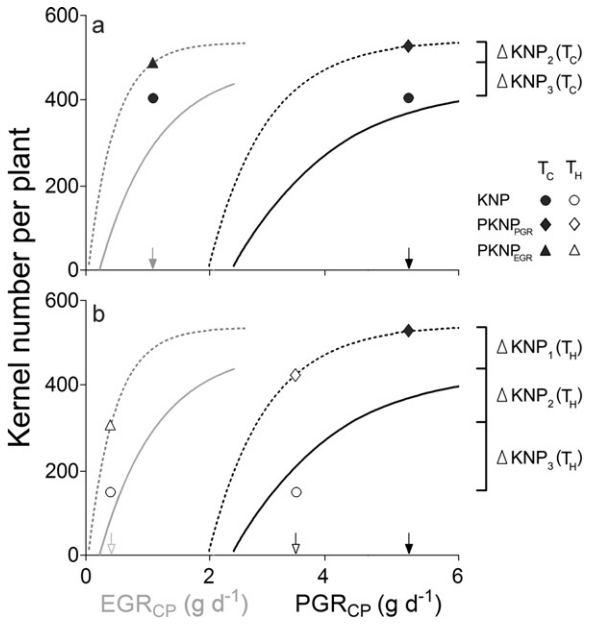

Fig. 1. Schematic representation of mean (filled line) and potential (dotted line) response of kernel number per plant (KNP) to plant (PGR_{CP}, dark lines) and ear (EGR_{CP}, gray lines) growth rates during the critical period for kernel set in (a) non-heated and (b) heated plots. Symbols represent kernel number values observed and calculated for any plant in the stand. For this plant, final KNP (circles) was registered at physiological maturity, and two potential KNPs ($PKNP_n$) values were calculated according to observed PGR_{CP} ($PKNP_{PGR}$, rhombus) and EGR_{CP} ($PKNP_{EGR}$, triangle) values. Arrows indicate observed PGR_{CP} (black arrows) and EGR_{CP} (gray arrows) values in heated (empty arrows) and non-heated (closed arrows) plots. The parameters obtained from the 99th quantile regression were used for calculating these theoretical values. The gap between $PKNP_{PGR}$ and KNP was attributed to three source of loss, which were related to PGR_{CP} reductions [$\Delta KNP_1 = 0$ for control plots and $\Delta KNP_{PGR} = PKNP_{PGR}(T_H) - PKNP_{PGR}(T_C)$ for heated plots], to changes in biomass partitioning to the ear ($\Delta KNP_2 = PKNP_{EGR} - PKNP_{PGR}$) and to constraints not directly related to assimilate allocation to the ear ($\Delta KNP_3 = KNP - PKNP_{EGR}$).

For each tagged plant we estimated two PKNP values, one considering its PGR_{CP} ($PKNP_{PGR}$) and the other one considering its EGR_{CP} ($PKNP_{EGR}$). These values were averaged for each plot and mean values were used for the computation of different sources of variation in KNP (ΔKNP_n) between $PKNP_{PGR}$ and KNP (Fig. 1).

The first source (ΔKNP_1) represented the decrease in PKNP due to PGR_{CP} reductions. It was null for control plots [$\Delta KNP_1(T_C) = 0$] and computed as in Eq. (3) for heated plots.

$$\Delta \mathrm{KNP}_1(T_H) = \mathrm{PKNP}_{\mathrm{PGR}}(T_H) - \mathrm{PKNP}_{\mathrm{PGR}}(T_C) \quad (3)$$

The second source of variation (ΔKNP_2) was attributable to changes in biomass partitioning to the ear. It was computed for each treatment combination as in Eq. (4):

$$\Delta \mathrm{KNP}_2 = \mathrm{PKNP}_{\mathrm{EGR}} - \mathrm{PKNP}_{\mathrm{PGR}} \quad (4)$$

The third source of variation (ΔKNP_3) represented the decrease in PKNP not related to assimilate allocation to the ear. It was computed for each treatment combination as in Eq. (5):

$$\Delta KNP_3 = KNP - PKNP_{EGR} \quad (5)$$

Heat effect on each source of variation in KNP was estimated as the difference between the ΔKNP_n obtained for heated [ΔKNP_n (T_H)] and non-heated [ΔKNP_n (T_C)] plots.

Mean values of each variable (measured and estimated) were averaged for each plot. ANOVA analysis was used to evaluate the effects of treatments and their interactions, and a *t*-test was applied to determine significant differences ($P < 0.05$) among means. Linear regression was used to test the relationship between EGR_{CP} and PGR_{CP}. The relationship between ΔKNP_2 and the partitioning index was fitted through the previously described curvilinear model [Eq. (1)].

3. Results

3.1. Growing conditions during the critical period for kernel set

Detailed information on meteorological conditions during experiments can be found in Rattalino Edreira et al. (2011). Briefly, mean air temperatures around flowering (ca. 30 d centered at silking of control plots) were similar between experimental years (25.5 °C for Exp_1 and 25.8 °C for Exp_2) but differed slightly between studied periods (24.6 °C for GS_1 and 26.1 °C for GS_2, averaged across experiments). Cumulative incident photosynthetically active radiation values during this period were higher in Exp_1 (277 MJ m^{-2}) than in Exp_2 (239 MJ m^{-2}). Difference between studied periods was also registered for this variable (239 MJ m^{-2} for GS_1 and 278 MJ m^{-2} for GS_2).

Heating increased air temperature at ear level during the treatment period, especially around midday (see Fig. 1 in Rattalino Edreira et al., 2011). Differences in this variable between heated and control plots were 4.61 °C from 1100 to 1600 h and 0.33 °C for the rest of the day (averaged across GS × H combinations and experiments). Mean daily absolute maximum air temperature at ear height was 35.2 ± 3.5 °C for heated plots and 30.2 ± 3.3 °C for control plots across all treatment combinations. Within each experiment, the intensity of heat stress was similar for each GS × H combination, but it was larger for Exp_1 (36 °C) than for Exp_2 (35.3 °C). Heating caused a gradual increase in organs temperature across the canopy (see Fig. 1 in Rattalino Edreira and Otegui, 2012). This trait was larger for the uppermost organs (i.e. tassel, uppermost leaves) than for the lowermost ones (i.e. basal internodes).

3.2. PGR_{CP}, EGR_{CP} and biomass partitioning to the ear

PGR_{CP} differed ($P < 0.001$) among hybrids, independently of temperature regimes (Table 1). PGR_{CP} in non-heated plots was larger for the Te hybrid than for the TeTr and Tr hybrids (4.7, 4.1 and 3.8 g $plant^{-1}$ d^{-1}, respectively; averaged across experiments and studied periods). Heat stress reduced PGR_{CP} ($P < 0.001$) between −25% and −52% across all treatment combinations, but the magnitude of this effect was similar among genotypes and between studied periods.

EGR_{CP} followed the trend described for PGR_{CP} in non-heated plots. In this condition, the Te hybrid had larger EGR_{CP} values than TeTr and Tr hybrids (0.90, 0.81 and 0.83 g d^{-1}, respectively; averaged across experiments and studied periods), but these differences were not significant. Heat stress reduced EGR_{CP} ($P < 0.001$, Table 1), and this negative effect was similar between studied periods but not among hybrids. EGR_{CP} reductions were smaller for the Tr hybrid (−36% respect to non-heated plots, averaged across experiments and studied periods) than for the Te (−54%) and the TeTr (−52%) hybrids.

For each hybrid, observed variation in EGR_{CP} was partly explained by the variation in PGR_{CP} ($r^2 \geq 0.45$, $n = 72$, $P < 0.001$, Fig. 2). The quotient between these variables (i.e. partitioning index, Table 1) was similar among hybrids and between studied periods in non-heated plots (0.19 for Te, 0.21 for TeTr and 0.23 for Tr hybrid; averaged across experiments and studied periods), but heat stress affected biomass partitioning to the ear (Table 1). The significant ($P = 0.004$) GS × H × TR interaction computed for this trait indicated that (i) PI was reduced by heating during the post-silking period independently of genotypes (−29% for Te, −34% for TeTr and −26% for Tr respect to non-heated plots; averaged across experiments), and (ii) heating before silking had a positive effect on PI for the Tr hybrid (57% for GS_1 respect to non-heated plots; averaged across experiments), but an opposite effect of early heating was detected for the Te (−8%) and TeTr (−13%) hybrids.

3.3. Mean response of KNP (least squares regression)

Final KNP was explained by the variation in both PGR_{CP} and EGR_{CP} (Fig. 3), but the latter was always a better predictor of KNP ($r^2 > 0.51$ for KNP-EGR_{CP} relationship, Table 2) than the former ($r^2 > 0.33$ for KNP-PGR_{CP} relationship). For each hybrid, curvilinear models [Eq. (1)] fitted by means of least square regression represented the mean response of KNP to changes in PGR_{CP} and EGR_{CP} caused by all treatments combinations (Exp × GS × TR). Some parameters of these models differed ($P < 0.05$) among hybrids (Table 2, Fig. 3).

The maximum number of kernels set at high PGR_{CP} (i.e. parameter a_{mean}, Table 2) tended to be smaller for the TeTr hybrid (458 KNP) than for the Te (488 KNP) and Tr (508 KNP) hybrids, but no significant difference was detected among them. A similar trend was registered for this parameter when the KNP-EGR_{CP} relationship was established (Table 2), and hybrids differed ($P < 0.05$) in the a_{mean} parameter of this relationship (Te = Tr > TeTr). The response of KNP to increments in PGR_{CP} or in EGR_{CP} (i.e. parameter b_{mean}) did not differ among hybrids, but genotypic differences could be observed for the PGR_{CP} threshold value for kernel set (i.e. parameter c_{mean}). This trait was smaller for the Tr hybrid (0.41 g d^{-1}) than for the Te (1.87 g d^{-1}) and TeTr (1.75 g d^{-1}) hybrids. Similarly, EGR_{CP} threshold value for kernel set was smaller for the Tr hybrid (0.14 g d^{-1}) than for the Te (0.19 g d^{-1}) and TeTr (0.2 g d^{-1}) ones.

3.4. Potential response of KNP (99th quantile regression)

The PKNP set at high PGR_{CP} (i.e. parameter a_{pot}) was smaller for the TeTr hybrid (463 PKNP) than for the Te (595 PKNP) and Tr (639 PKNP) ones. A similar trend was observed among genotypes for this parameter (490, 611 and 663 PKNP for the TeTr, Te and Tr hybrids, respectively) when the PKNP-EGR_{CP} relationship was analyzed. The response of PKNP to increments in PGR_{CP} or in EGR_{CP} (i.e. parameter b_{pot}) was similar among hybrids. Contrary, parameter c_{pot} (i.e. threshold value for kernel set) differed ($P < 0.05$) among genotypes when both KNP-PGR_{CP} and KNP-EGR_{CP} relationships were set (Table 2). Coefficients of determination (r^1) were similar among hybrids for each relationship, indicating a similar goodness of fit of established curvilinear models [Eq. (2)] among them.

3.5. PKNP and sources of loss in kernel numbers

Potential kernel number per plant (PKNP) expected from observed PGR_{CP} values (i.e. $PKNP_{PGR}$) differed among hybrids ($P < 0.001$), but not between experiments and studied periods when

Table 1
Plant and ear growth rates during the critical period for kernel set and biomass partitioning to the ear.

Exp[a]	GS	H	TR	PGR_{CP} ($g\,d^{-1}$)	EGR_{CP} ($g\,d^{-1}$)	PI
Exp_1	GS_1	Te	T_C	5.71	1.02	0.17
			T_H	3.23	0.43	0.14
		TeTr	T_C	4.19	0.78	0.18
			T_H	2.49	0.40	0.16
		Tr	T_C	4.50	0.85	0.18
			T_H	2.20	0.81	0.35
	GS_2	Te	T_C	5.38	0.99	0.18
			T_H	3.07	0.34	0.11
		TeTr	T_C	5.22	0.90	0.17
			T_H	3.52	0.45	0.13
		Tr	T_C	4.07	0.96	0.24
			T_H	2.90	0.54	0.19
Exp_2	GS_1	Te	T_C	4.21	0.92	0.22
			T_H	2.38	0.50	0.22
		TeTr	T_C	3.55	1.03	0.29
			T_H	2.02	0.51	0.25
		Tr	T_C	3.73	0.81	0.22
			T_H	1.81	0.48	0.27
	GS_2	Te	T_C	3.63	0.69	0.19
			T_H	2.37	0.37	0.15
		TeTr	T_C	3.58	0.55	0.15
			T_H	2.69	0.23	0.09
		Tr	T_C	2.85	0.71	0.27
			T_H	1.93	0.31	0.19
Exp				0.001[b]	0.026	ns
GS				ns	0.007	0.006
H				0.002	ns	0.001
TR				<0.001	<0.001	ns
Exp × GS				0.017	0.012	ns
Exp × H				ns	0.046	ns
Exp × TR				0.005	ns	ns
GS × H				0.014	ns	ns
GS × TR				0.003	ns	0.000
H × TR				ns	0.022	0.021
Exp × GS × H				ns	0.015	0.034
Exp × GS × TR				ns	0.033	ns
Exp × H × TR				ns	0.022	ns
GS × H × TR				ns	ns	0.004

[a] Exp: experiment; GS: growth stage; H: Hybrid; TR: temperature regime; EGR_{CP}: ear growth rate during the critical period; PGR_{CP}: plant growth rate during the critical period; PI: partitioning index ($EGR_{CP}\ PGR_{CP}^{-1}$); Te: temperate; Tr: tropical; TeTr: Te × Tr; T_C: non-heated control; T_H: heated.
[b] P values of main and interaction effects for which at least one variable was detected as significant; ns, not significant ($P > 0.05$).

the analysis considered only the non-heated plots. $PKNP_{PGR}$ values were smaller for the TeTr hybrid (453 $PKNP_{PGR}$; averaged of control plots across experiments and studied periods) than for the Te (588 $PKNP_{PGR}$) and Tr (610 $PKNP_{PGR}$) ones. Similar results were obtained for the PKNP expected from observed EGR_{CP} values (i.e. $PKNP_{EGR}$). Both $PKNP_{PGR}$ and $PKNP_{EGR}$ were close to the expected absolute PKNP at high PGR_{CP} (i.e. parameter a_{pot}; previously described in Section 3.4).

Heat stress reduced $PKNP_{PGR}$ ($P < 0.001$, Table 3) in all treatment combinations, but the significant ($P = 0.012$) GS × TR interaction detected for this trait indicated that this negative effect was stronger for heating before silking (−23% of control plots, averaged across hybrids and experiments) than for heating after silking (−10%) in both experiments. Hybrids did not differ in the response to heating for this trait. $PKNP_{EGR}$ was always severely affected by heat stress ($P < 0.001$, Table 3), but the magnitude of this effect

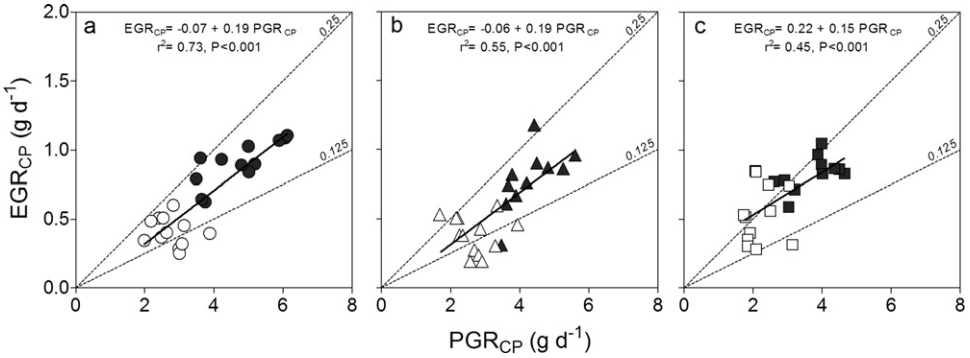

Fig. 2. Relationship between ear (EGR_{CP}) and plant (PGR_{CP}) growth rates during the critical period for kernel set of (a) temperate, (b) temperate × tropical, and (c) tropical hybrids exposed to heated (open symbols) and non-heated (close symbols) conditions around flowering. Each symbol represents the mean of nine plants within each replicate and experimental year. Dotted lines represent the 0.125 and 0.25 ratios between variables.

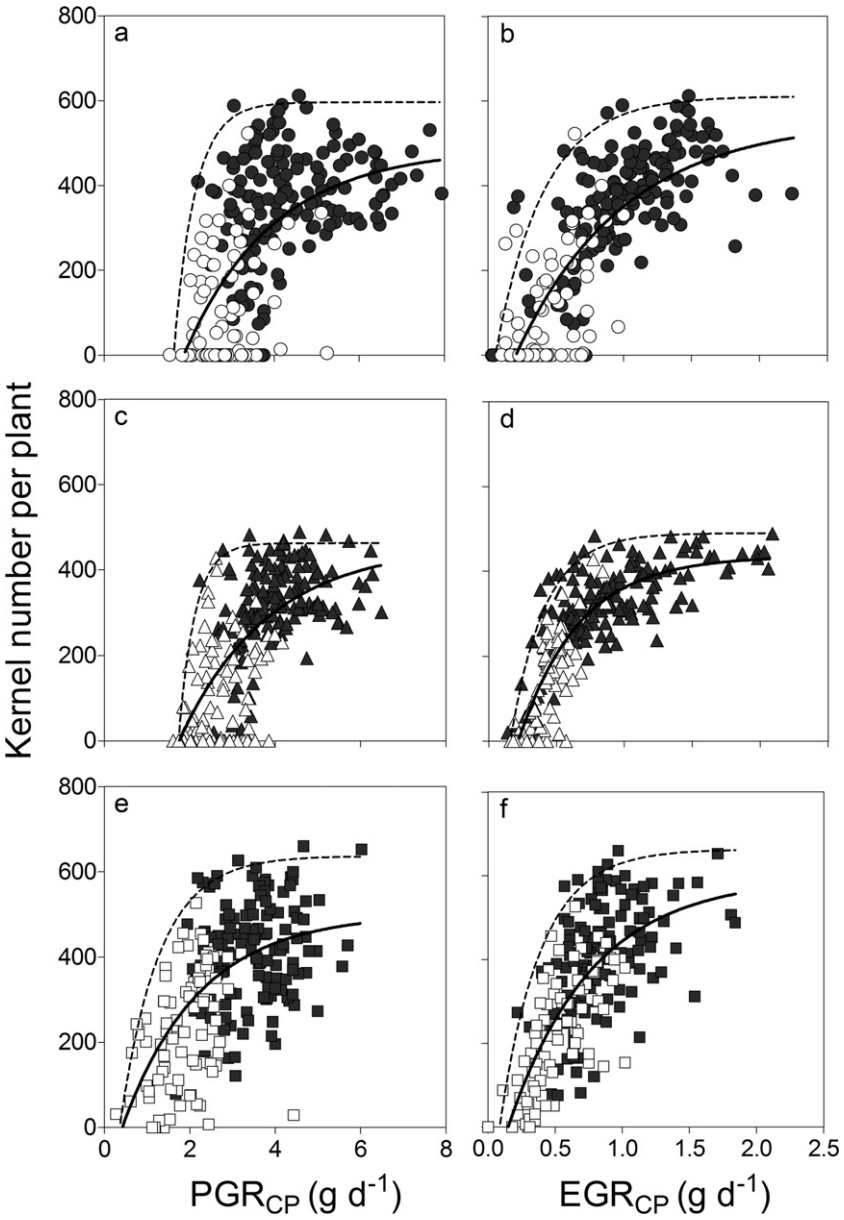

Fig. 3. Response of kernel number per plant to plant (PGR_{CP}; figures a, c and e) and ear (EGR_{CP}; figures b, d and f) growth rates during the critical period for kernel set of three maize hybrids of different genetic background exposed to heated (open symbols) and non-heated (close symbols) conditions around flowering. Hybrids of temperate (a and b), temperate × tropical (c and d) or tropical (e and f) background were surveyed during two experimental years. For each hybrid, points represent individual plant data and lines indicate models fitted to the uppermost 99th quantile (dotted line) or to the center of data distributions (filled line). Parameters of the curvilinear relationships are detailed in Table 2.

was larger for heating during the post-silking period (−38% for GS_2) than for heating during the pre-silking period (−22% for GS_1). Additionally, hybrids differed in the response to above-optimum temperatures for this trait. The TeTr hybrid tended to exhibit a larger reduction in $PKNP_{EGR}$ due to heating (−38% of control plots, averaged across experiments and studied periods) than the Te (−29%) and Tr (−22%) hybrids.

Final KNP in non-heated plots was similar between growth stages during Exp_1 (335 KNP for GS_1 and 350 KNP for GS_2, averaged across hybrids), but this trait was more extensively reduced for GS_2 (243 KNP) than for GS_1 (411 KNP) during Exp_2 (Table 3). Final KNP did not differ among hybrids in non-heated plots (313, 326 and 368 KNP for the Te, TeTr and Tr hybrids, respectively), however, the gap between actual and potential KNP (i.e. KNP – $PKNP_{PGR}$) was smaller for the TeTr (−140 KNP) hybrid than for the Te (−264 KNP) and Tr (−242 KNP) hybrids. In this condition, the largest proportion of this total loss in KNP was attributable to constraints not directly related to assimilate allocation to the ear (i.e. ΔKNP_3, 84%, averaged of control plots across all treatment combinations) and the rest (16%) to changes in biomass partitioning to the ear (i.e. ΔKNP_2).

Heat stress around flowering had a severe effect on KNP, especially when it was performed after silking (Table 3). Though $PKNP_{PGR}$ was smaller in heated plots than in the non-heated ones, the gap between actual and potential KNP was larger for the former (−334 KNP, averaged across all treatment combinations) than for the latter (−215 KNP). The contribution of each source of loss to the decrease in PKNP was 30% for ΔKNP_1, 16% for ΔKNP_2, and 54% for ΔKNP_3 (averaged of heated plots across all treatment combinations).

ANOVA analysis (Table 3) clearly indicated that computed losses in KNP that could be attributable exclusively to heat effects (i.e.

Table 2
Parameters of curvilinear models fitted to the response of kernel number per plant (KNP) or potential kernel number per plant (PKNP) to plant (PGR_{CP}) and ear (EGR_{CP}) growth rates during the critical period. Models correspond to the least square regression (KNP) or to the 99th quantile regression (PKNP).

Relationship	Hybrid	Least square regression			
		a_{mean}	b_{mean}	c_{mean}	r^2
KNP-PGR_{CP}	Te	488 a	2.1 a	1.87 a	0.47
	TeTr	458 a	2.1 a	1.75 a	0.46
	Tr	502 a	1.8 a	0.41 b	0.34
KNP-EGR_{CP}	Te	557 a	0.8 a	0.19 a	0.65
	TeTr	437 b	0.5 a	0.20 a	0.76
	Tr	596 a	0.6 a	0.14 b	0.51
Relationship	Hybrid	99th quantile regression			
		a_{pot}	b_{pot}	c_{pot}	r^1
PKNP-PGR_{CP}	Te	595 a	0.5 a	1.77 a	0.15
	TeTr	463 b	0.4 a	1.73 a	0.19
	Tr	639 a	1.0 a	0.43 b	0.22
PKNP-EGR_{CP}	Te	611 a	0.4 a	0.06 b	0.36
	TeTr	490 b	0.2 a	0.16 a	0.42
	Tr	663 a	0.3 a	0.09 ab	0.26

Different letters within each column and relationship indicate significant differences ($P<0.05$) among hybrids. All models fitted by least square regression were significant at $P<0.001$

HE_1, HE_2 and HE_3) were more affected by the time of stress (i.e. GS, $P \leq 0.05$) than by the genotypes (i.e. H, $P>0.10$). However, genotypic differences ($P=0.09$) were detected when all sources of heat-induced kernel loss (i.e. $HE_1+HE_2+HE_3$) were analyzed together (analysis not shown). Total absolute losses in KNP attributable exclusively to heat stress were larger for the Te hybrid (−245 KNP, averaged across experiments and studied periods) than for the TeTr (−210 KNP) and Tr (−165 KNP) hybrids, and these losses represented KNP reductions of 77%, 69% and 44%, respectively, as compared to their non-heated counterparts. Averaging across experiments and studied periods, the observed differences in kernel loss between Te and Tr hybrids were attributable to changes in biomass partitioning to the ear [i.e. HE_2 (Te) − HE_2 (Tr) = −20 KNP] and to constraints not directly related to assimilate allocation to the ear [i.e. HE_3 (Te) − HE_3 (Tr) = −60 KNP], but not to changes in PGR_{CP} [i.e. HE_1 (Te) − HE_1 (Tr) = 0 KNP].

Generally, heating before silking had a larger effect on KNP due to PGR_{CP} reductions (i.e. HE_1) than heating after silking (Table 3). Contrary, late heating (i.e. GS_2) caused larger loss in KNP due to changes in biomass partitioning to the ear (i.e. HE_2) than early heating (i.e. GS_1). Moreover, the beneficial effect of early heating on biomass partitioning to the ear detected for the Tr hybrid (previously described in Section 3.2) enhanced ΔKNP_2 values, and thus, positive HE_2 values could be observed for this genotype when heating was performed during the pre-silking period. Established relationship between ΔKNP_2 and PI ($r^2=0.85$, Fig. 4) identified

Table 3
Potential ($PKNP_{PGR}$ and $PKNP_{EGR}$) and final kernel numbers per plant (KNP), and sources of loss in kernel numbers (ΔKNP_n and HE_n).

Exp[a]	GS	H	TR	$PKNP_{PGR}$	$PKNP_{EGR}$	KNP	Source of loss					
							ΔKNP_1	HE_1	ΔKNP_2	HE_2	ΔKNP_3	HE_3
Exp_1	GS_1	Te	T_C	591	525	351	0		−66		−174	
			T_H	542	380	140	−49	−49	−162	−95	−240	−66
		TeTr	T_C	460	431	320	0		−28		−112	
			T_H	387	292	125	−72	−72	−95	−67	−167	−55
		Tr	T_C	627	585	334	0		−42		−251	
			T_H	533	558	339	−95	−95	25	67	−219	32
	GS_2	Te	T_C	593	512	337	0		−82		−175	
			T_H	554	316	23	−40	−40	−238	−156	−293	−119
		TeTr	T_C	463	457	322	0		−6		−135	
			T_H	457	311	130	−5	−5	−146	−140	−182	−46
		Tr	T_C	625	615	392	0		−10		−223	
			T_H	589	472	183	−36	−36	−117	−107	−289	−67
Exp_2	GS_1	Te	T_C	586	525	392	0		−61		−133	
			T_H	417	425	108	−169	−169	8	69	−316	−184
		TeTr	T_C	431	452	375	0		21		−77	
			T_H	229	337	144	−202	−202	108	87	−193	−116
		Tr	T_C	615	588	464	0		−26		−124	
			T_H	477	469	200	−138	−138	−8	18	−268	−144
	GS_2	Te	T_C	580	495	213	0		−85		−282	
			T_H	477	346	39	−102	−102	−132	−47	−306	−24
		TeTr	T_C	458	337	234	0		−121		−103	
			T_H	406	121	13	−52	−52	−284	−164	−109	−5
		Tr	T_C	573	570	283	0		−3		−287	
			T_H	481	349	93	−92	−92	−132	−129	−256	31
Exp				0.001[b]	ns	ns	0.001	<0.001	ns	ns	ns	ns
GS				0.011	0.012	0.004	<0.001	<0.001	0.010	0.001	ns	0.050
H				<0.001	<0.001	0.002	ns	ns	0.028	ns	<0.0001	ns
TR				<0.001	<0.001	<0.001	<0.001	–	<0.001	–	<0.0001	–
Exp × GS				ns	0.034	0.026	0.024	0.024	ns	ns	ns	0.006
Exp × H				ns	0.032	ns	ns	ns	ns	ns	0.018	ns
Exp × TR				<0.001	ns	ns	<0.001	–	ns	–	ns	–
GS × TR				<0.001	0.012	ns	<0.001	–	<0.001	–	0.027	–
Exp × GS × H				ns	0.025	ns	ns	ns	0.018	ns	ns	ns
Exp × GS × TR				ns	ns	0.012	ns	–	ns	–	<0.001	–

[a] Exp: experiment; GS: growth stage; H: Hybrid; TR: temperature regime; $PKNP_{PGR}$, PKNP estimated from plant growth rate during the critical period for kernel set (PGR_{CP}); $PKNP_{EGR}$, PKNP estimated from ear growth rate during the critical period for kernel set (EGR_{CP}); ΔKNP_1, loss in PKNP due to PGR_{CP} reduction; HE_1, heat effect on ΔKNP_1; ΔKNP_2, loss in PKNP due to changes in biomass partitioning to the ear; HE_2, heat effect on ΔKNP_2; ΔKNP_3, loss in PKNP due to constraints not directly related to assimilate allocation to the ear; HE_3, heat effect on ΔKNP_3; Te: temperate; Tr: tropical; TeTr: Te × Tr; T_C: non-heated control; T_H: heated;

[b] P values of main and interaction effects for which at least one variable was detected as significant; ns: not significant ($P>0.05$).

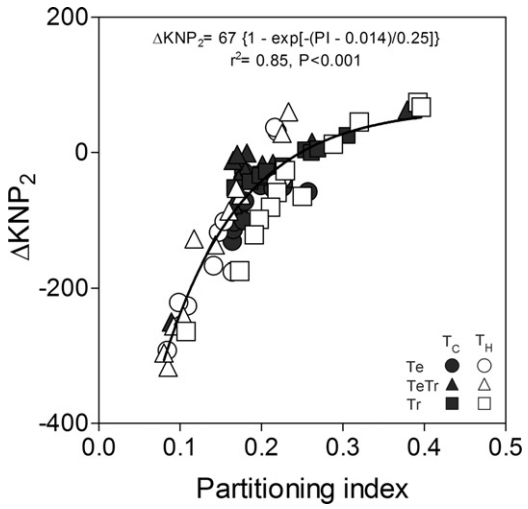

Fig. 4. Relationship between kernel number per plant loss due to changes in biomass partitioning to the ear (ΔKNP_2) and partitioning index ($EGR_{CP}\ PGR_{CP}^{-1}$) of temperate (Te), temperate × tropical (TeTr), and tropical (Tr) hybrids exposed to heated (open symbols) and non-heated (close symbols) conditions around flowering. Each symbol represents the mean of nine plants in each experimental year.

an index threshold value of 0.25 for avoiding kernel loss due to reduced biomass partitioning to the ear. Negative effect of heating on KNP due to constraints not directly related to assimilate allocation to the ear (i.e. HE_3) was larger for post-silking (−77 KNP for GS_2) than for pre-silking (−30 KNP for GS_1) heating during Exp_1, but the opposite trend was found during Exp_2 because of the large reduction in ΔKNP_3 observed for GS_2 of non-heated plots (Table 3).

4. Discussion

Our research expanded the reach of a previous study (Rattalino Edreira et al., 2011), which was based predominantly on the evaluation of developmental characteristics (e.g. anthesis-silking interval, potential ear size, number of exposed silks) for the interpretation of different sources of loss in maize kernel set. In the current paper we focused on the effects of heat stress on kernel number determination of maize hybrids that differ in their tolerance to heat stress (Rattalino Edreira and Otegui, 2012). Genotypic differences were detected for most studied traits in non-heated plots. The $PKNP_{PGR}$ calculated for each genotype in this condition was close to the maximum KNP expected at high resource availability per plant (i.e. parameter a_{pot}), which suggested that most individuals in non-heated plots experienced high PGR_{CP} (Andrade et al., 1999), and were very uniform in size among them (Maddonni and Otegui, 2004). Results also indicated that differences among hybrids in $PKNP_{PGR}$ were related to genotypic differences in parameter a_{pot}, but not to differences in the potential response of KNP to PGR_{CP} increments (i.e. parameter b_{pot}) or to differences in the minimum PGR_{CP} threshold for kernel set (i.e. parameter c_{pot}). Increments in a_{pot} may be related to enhanced kernel set at high PGR_{CP} and/or to enhanced potential ear size (i.e. florets per ear). Breeding did not significantly increase yield potential per plant of temperate American hybrids (Duvick and Cassman, 1999; Duvick, 2005), but there are evidences of a positive trend in these traits (kernel set at high PGR_{CP} and/or potential ear size) for Canadian (Tollenaar et al., 1992) and Argentine hybrids (Echarte et al., 2004; Luque et al., 2006). In current research, the TeTr hybrid had the lowest $PKNP_{PGR}$ value, but its final KNP was similar to those registered for the other hybrids in non-heated plots. This was the result of its low gap between actual and potential kernel numbers, largely attributable to its reduced kernel loss due to constraints not directly related to assimilate allocation to the ear (i.e. ΔKNP_3) respect to the other hybrids. The highest coefficient of determination of the KNP-EGR_{CP} relationship observed for the TeTr hybrid confirms this result (Table 3). Genotypic differences in the magnitude of ΔKNP_3 may be inherent to each genotype because hybrids grew under similar environmental conditions around flowering (Rattalino Edreira and Otegui, 2012) and there were no genotypic differences in traits related to flowering events among tested hybrids, such as flowering dynamic or anthesis-silking interval (Rattalino Edreira et al., 2011).

Variations in PGR_{CP} or in EGR_{CP} gave an acceptable explanation of the observed variation in KNP, in agreement with previous research on maize kernel number determination on an individual plant basis under abiotic stress (Echarte and Tollenaar, 2006; D'Andrea et al., 2008; Rossini et al., 2011). These relationships indicated that kernel losses due to heating were mediated, at least in part, by assimilates production and their supply to the ear around flowering, as was previously demonstrated for one single-cross hybrid of temperate background heated during GS_1 (Cicchino et al., 2010b). Heat stress around flowering severely reduced KNP, and this negative effect was mainly related to PGR_{CP} reductions (i.e. ΔKNP_1), as reported for water and nitrogen deficiencies (Muchow and Davis, 1988; Uhart and Andrade, 1995; Andrade et al., 2002). In our experiments, genotypes had a similar response in kernel loss due to PGR_{CP} reductions induced by heating (i.e. HE_1), largely attributable to the similar effect of heating on PGR_{CP} and the similar response pattern of KNP to variations in PGR_{CP} (i.e. parameter b_{pot}) among them. Despite these results, two considerations may be drawn from the curvilinear relationship between KNP and PGR_{CP} in maize. First, the negative effect of heating on kernel set depends on the absolute PGR_{CP} reduction. Second, it also depends on the PGR_{CP} range explored across the KNP-PGR_{CP} relationship. In other words, genotypic differences in this source of kernel loss may be related to genotypes ability to sustain plant growth under heat stress, but also to plant growth conditions prior to stress. The latter suggests that crop management practices that enhance PGR_{CP} prior to stress, such as reduced stand density and adequate nutrients provision, may contribute to diminish this source of kernel loss.

A relevant output of current research was the clear assessment of KNP losses due to changes in biomass partitioning to the ear (i.e. ΔKNP_2), and its robust relationship with an index (PI) representative of biomass allocation to this organ during the critical period for kernel set (Fig. 4), which held across all tested treatments (i.e. temperature regimes, studied periods and hybrids). This relevant finding represents a step forward respect to simple KNP-PGR_{CP} and KNP-EGR_{CP} relationships explored until now (Echarte and Tollenaar, 2006; Pagano and Maddonni, 2007; D'Andrea et al., 2008; Cicchino et al., 2010b; Rossini et al., 2011). The relationship between ΔKNP_2 and PI allowed us to identify a critical PI value for avoiding these kernel losses. It also improved previous estimates on KNP variations, either based on changes in PGR_{CP} ($r^2 \leq 0.74$ in op.cit.) or on EGR_{CP} ($r^2 \leq 0.75$ in op.cit.), though improved robustness of our results ($r^2 = 0.85$) cannot be compared with other research due to the novelty of our approach. Contrary to the other sources of kernel loss (i.e. ΔKNP_1 and ΔKNP_3), positive ΔKNP_2 values were registered. These values were all related to GS_1 and mostly to heated plots, though negative values prevailed in most treatment combinations. This response may be partially attributed to reduced apical dominance effects on biomass allocation to ear growth of heated plots due to large negative effects of the stress on tassel growth during this stage, as already reported by Cichino et al. (2010b). This trend disappeared for heat stress during GS_2, when tassel growth has been completed (i.e., no effect on apical

dominance) and the negative effects of above-optimum temperature on kernel set caused a permanent reduction in ear sink strength with the concomitant decline in assimilate allocation to this organ. Additionally, low PI values registered in our study were probably associated with the late sowing date (Otegui et al., 1995) used for achieving adequate differences in temperature between control and heated plots. Therefore, the magnitude of kernel losses attributable to changes in biomass partitioning to the ear observed in our plants may be higher than those expected from plants cropped in a similar environment but in early sowings (Pagano and Maddonni, 2007; Rossini et al., 2011), which usually have higher PI values than ours.

Although heat stress reduced assimilates availability per plant, biomass partitioning to the ear did not vary markedly, and a comparatively low negative effect of heating was registered for ΔKNP_2. This response, also reported by Cicchino et al. (2010b), was opposite to that expected from water (Hall et al., 1981; NeSmith and Ritchie, 1992) or nitrogen deficiencies (Uhart and Andrade, 1995; D'Andrea et al., 2008), for which assimilate supply to the ear decreased sharply when resource availability per plant declined severely before anthesis. This response has been chiefly attributed to the dominated nature of this organ as compare to the tassel and the uppermost internodes (Otegui, 1997). Low effects of heating around flowering on biomass partitioning to the ear have been attributed (Cicchino et al., 2010a) to the fact that many times this constraint has a larger effect on dominant (tassel and uppermost leaves) than on dominated (ears) organs (Rattalino Edreira and Otegui, 2012), and may be catastrophic when tassels are already exposed and starting anthesis (Herrero and Johnson, 1980). These effects may reduce the sink strength of dominant organs, and thus their competition for assimilates with the ear. Enhanced PIs values (current research) and reduced anthesis-silking intervals (Rattalino Edreira et al., 2011) of heated plots support these contentions. Positive effects of restricted tassel growth may be most important among genotypes with large size and excessive foliage (Grogan, 1956; Hunter et al., 1969), like tropical maize (Fischer and Palmer, 1984). This speculation could not be verified from our results, because tassel growth of all hybrids was severely affected by pre-anthesis heating (Rattalino Edreira et al., 2011) and all have similar plant size at flowering (Rattalino Edreira and Otegui, 2012).

The second most important source of kernel loss that could be attributable exclusively to heat effects was associated with limiting factors that are not directly related to assimilate allocation to the ear (i.e. ΔKNP_3). Several studies on heat stress around flowering identified pollination, fertilization and kernel set as the most heat-sensitive reproductive processes in cereals (Barnabás et al., 2008). Pollination failures due to above-optimum temperatures in maize have been associated with negative effects on pollen shed (Schoper et al., 1987) and pollen viability (Herrero and Johnson, 1980; Schoper et al., 1986; Mitchell and Petolino, 1988) but not with silks emergence (Rattalino Edreira et al., 2011) or silks receptivity (Dupuis and Dumas, 1990). In our experiments, the observed effect of heating on ΔKNP_3 might not be attributable to pollination/fertilization failures because of the manual addition of fresh pollen in heated pots and silk growth arrest after this procedure. Additionally, previous evidence indicated that kernel loss may not be related to reduced floret differentiation and failure to expose a silk from a developed floret, as above-optimum temperatures have little effect on these processes (Rattalino Edreira et al., 2011). Collectively, these evidences allowed us to speculate that kernel abortion may be the main source of variation in ΔKNP_3 due to heating. This source of kernel loss was always larger for the Te hybrid than for hybrids with tropical genetic background (TeTr and Tr hybrids).

5. Conclusions

Heat stress had a severe effect on plant and ear growth rates during the critical period for kernel set, but biomass partitioning to the ear was less affected. Key issues emerging from this study are (i) the development of a novel approach based on ecophysiological traits for assessing sources of kernel loss in field conditions, and (ii) the identification of traits associated with enhanced heat-tolerance among genotypes of contrasting genetic background. The former allowed us to identify reductions in PGR_{CP} as the main source of kernel loss attributable exclusively to heat effects, followed by losses associated with constraints not directly related to assimilate allocation to the ear and to biomass partitioning to the ear. Enhanced tolerance to heat stress of the tropical genotype was mainly associated with reduced kernel abortion (i.e. third source of loss). The identification of these traits had not been possible by means of the independent analysis of the response of KNP to PGR_{CP} and EGR_{CP}. This highlights the importance of the new approach as an aid to genotype selection to be used in breeding programs.

Acknowledgements

Authors wish to thank Luis I. Mayer, Alejandra Seco, Paula Aguirre, Damian Sammarro, Maxime Puech and Clémence Mercier for their help with field work. We also thank Santiago L. Poggio for his valuable comments and suggestions on the statistical analysis. Juan I. Rattalino Edreira held a grant for graduate studies of the National Council for Research (CONICET), and Maria E. Otegui is a member of CONICET. This work was financed by the CONICET (project PID 00125), UBA (project UBACYT 00454), FONCYT (project PICT 0239) and the Regional Fund for Agricultural Technology (FONTAGRO, project 8031).

References

Aluko, G.K., Fischer, K.S., 1988. The effect of changes of assimilate supply around flowering on grain sink size and yield of maize (*Zea mays* L) cultivars of tropical and temperate adaptation. Aust. J. Agric. Res. 38, 153–161.
Andrade, F.H., Echarte, L., Rizzalli, R., Della Maggiora, A., Casanovas, M., 2002. Kernel number prediction in maize under nitrogen or water stress. Crop Sci. 42, 1173–1179.
Andrade, F.H., Vega, C., Uhart, S., Cirilo, A., Cantarero, M., Valentinuz, O., 1999. Kernel number determination in maize. Crop Sci. 39, 453–459.
Barnabás, B., Jager, K., Feher, A., 2008. The effect of drought and heat stress on reproductive processes in cereals. Plant Cell Environ. 31, 11–38.
Cade, B.S., Terrell, J.W., Schroeder, R.L., 1999. Estimating effects of limiting factors with regression quantiles. Ecology 80, 311–323.
Cicchino, M., Rattalino Edreira, J.I., Otegui, M.E., 2010a. Heat stress during late vegetative growth of maize: effects on phenology and assessment of optimum temperature. Crop Sci. 50, 1431–1437.
Cicchino, M., Rattalino Edreira, J.I., Uribelarrea, M., Otegui, M.E., 2010b. Heat stress in field grown maize: response of physiological determinants of grain yield. Crop Sci. 50, 1438–1448.
Cheikh, N., Jones, R.J., 1994. Disruption of maize kernel growth and development by heat stress. Plant Physiol. 106, 45–51.
D'Andrea, K.E., Otegui, M.E., Cirilo, A., 2008. Kernel number determination differs among maize hybrids in response to nitrogen. Field Crops Res. 105, 228–239.
Daynard, T.B., Duncan, W.G., 1969. The black layer and grain maturity in corn. Crop Sci. 9, 473–476.
Dupuis, I., Dumas, C., 1990. Influence of temperature stress on in vitro fertilization and heat shock protein synthesis in maize (*Zea mays* L.) reproductive tissues. Plant Physiol. 94, 665–670.
Duvick, D.N., 2005. The contribution of breeding to yield advance in maize (Zea mays L.). Adv. Agron. 86, 83–145.
Duvick, D.N., Cassman, K.G., 1999. Post-green revolution trends in yield potential of temperate maize in the north-central United States. Crop Sci. 39, 1622–1630.
Echarte, L., Andrade, F.H., Vega, C.R.C., Tollenaar, M., 2004. Kernel number determination in argentinean maize hybrids released between 1965 and 1993. Crop Sci. 44, 1654–1661.
Echarte, L., Tollenaar, M., 2006. Kernel set in maize hybrids and their inbred lines exposed to stress. Crop Sci. 46, 870–878.
Fischer, K.S., Palmer, A.F.E., 1984. Tropical maize. In: Goldsworthy, P.R., Fisher, N.M. (Eds.), The Physiology of Tropical Field Crops. John Wiley & Sons, Chichester, England, pp. 213–248.

French, R.J., Schultz, J.E., 1984. Water use efficiency of wheat in a mediterranean-type environment I. The relation between yield, water use and climate. Aust. J. Agric. Res. 35, 743–764.
Grant, R., Jackson, B., Kiniry, J., Arkin, G., 1989. Water deficit timing effects on yield components in maize. Agron. J. 81, 61–65.
Grogan, C.O., 1956. Detasseling responses in corn. Agron. J. 48, 247–249.
Hall, A.J., Lemcoff, J.H., Trapani, N., 1981. Water stress before and during flowering in maize and its effects on yield, its components, and their determinants. Maydica 26, 19–38.
Herrero, M.P., Johnson, R.R., 1980. High temperature stress and pollen viability of maize. Crop Sci. 20, 796–800.
Hunter, R.B., Daynard, T.B., Hume, D.J., Tanner, J.W., Curtis, J.D., Kannenberg, L.W., 1969. Effect of tassel removal on grain yield of corn (*Zea mays* L.). Crop Sci. 9, 405–406.
Kiniry, J.R., Ritchie, J.T., 1985. Shade-sensitive interval of kernel number of maize. Agron. J. 77, 711–715.
Koenker, R., Machado, J.A.F., 1999. Goodness of fit and related inference processes for quantile regression. J. Am. Stat. Ass. 94, 1296–1310.
Luque, S.F., Cirilo, A.G., Otegui, M.E., 2006. Genetic gains in grain yield and related physiological attributes in Argentine maize hybrids. Field Crops Res. 95, 383–397.
Maddonni, G.A., Otegui, M.E., 2004. Intra-specific competition in maize: early establishment of hierarchies among plants affects final kernel set. Field Crops Res. 85, 1–13.
Mitchell, J.C., Petolino, J.F., 1988. Heat stress effects on isolated reproductive organs of maize. Plant Physiol. 133, 625–628.
Monfreda, C., Ramankutty, N., Foley, J.A., 2008. Farming the planet: 2 Geographic distribution of crop areas, yields, physiological types, and net primary production in the year 2000. Glob. Biogeochem. Cycles 22, GB1022.
Muchow, R.C., Davis, R., 1988. Effect of nitrogen supply on the comparative productivity of maize and sorghum in a semi-arid tropical environment II Radiation interception and biomass accumulation. Field Crops Res. 18, 17–30.
NeSmith, D.S., Ritchie, J.T., 1992. Effects of soil water-deficits during tassel emergence on development and yield component of maize (*Zea mays*). Field Crops Res. 28, 251–256.
Otegui, M.E., 1997. Kernel set and flower synchrony within the ear of maize: II Plant population effects. Crop Sci. 37, 448–455.
Otegui, M.E., Andrade, F.H., Suero, E.E., 1995. Growth, water use, and kernel abortion of maize subjected to drought at silking. Field Crops Res. 40, 87–94.
Otegui, M.E., Bonhomme, R., 1998. Grain yield components in maize I Ear growth and kernel set. Field Crops Res. 56, 247–256.
Otegui, M.E., Ruiz, R.A., Petruzzi, D., 1996. Modeling hybrid and sowing date effects on potential grain yield of maize in a humid temperate region. Field Crops Res. 47, 167–174.
Pagano, E., Cela, S., Maddonni, G.A., Otegui, M.E., 2007. Intra-specific competition in maize: Ear development flowering dynamics and kernel set of early-established plant hierarchies. Field Crops Res. 102, 198–209.
Pagano, E., Maddonni, G.A., 2007. Intra-specific competition in maize: early established hierarchies differ in plant growth and biomass partitioning to the ear around silking. Field Crops Res. 101, 306–320.
Parry, M., Rosenzweig, C., Iglesias, A., Fischer, G., Livermore, M., 1999. Climate change and world food security: a new assessment. Glob. Environ. Change. 9, 51–67.
R Development Core Team, 2011. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
Rattalino Edreira, J.I., Budakli Carpici, E., Sammarro, D., Otegui, M.E., 2011. Heat stress effects around flowering on kernel set of temperate and tropical maize hybrids. Field Crops Res. 123, 62–73.
Rattalino Edreira, J.I., Otegui, M.E., 2012. Heat stress in temperate and tropical maize hybrids: Differences in crop growth, biomass partitioning and reserves use. Field Crops Res. 130, 87–98.
Ritchie, S.W., Hanway, J.J., 1982. How a plant crop develops. Spec. Rep. 48. Iowa State Univ. of Sci. and Technol., Coop. Ext. Serv., Ames, IA.
Rossini, M.A., Maddonni, G.A., Otegui, M.E., 2011. Inter-plant competition for resources in maize crops grown under contrasting nitrogen supply and density: variability in plant and ear growth. Field Crops Res. 121, 373–380.
Schmidhuber, J., Tubiello, F.N., 2005. Global food security under climate change. Proc. Natl. Acad. Sci. U. S. A. 104, 19703–19708.
Schoper, J.B., Johnson, R.R., Lambert, R.J., 1982. Maize yield response to increased assimilate supply. Crop Sci. 22, 1184–1189.
Schoper, J.B., Lambert, R.J., Vasilas, B.L., 1986. Maize pollen viability and ear receptivity under water and high temperature stress. Crop Sci. 26, 1029–1033.
Schoper, J.B., Lambert, R.J., Vasilas, B.L., Westgate, M.E., 1987. Plant factors controlling seed set in maize. Plant Physiol. 83, 121–125.
Teixeira, E.I., Fischer, G., van Velthuizen, H., Walter, C., Ewert, F., 2011. Global hotspots of heat stress on agricultural crops due to climate change. Agric. For. Meteorol., in press.
Tollenaar, M., Dwyer, L.M., Stewart, D.W., 1992. Ear and kernel formation in maize hybrids representing three decades of grain yield improvement in Ontario. Crop Sci. 32, 432–438.
Uhart, S.A., Andrade, F.H., 1995. Nitrogen deficiency in maize: I Effects on crop growth, development, dry matter partitioning, and kernel set. Crop Sci. 35, 1376–1383.
Vega, C.R.C., Andrade, F.H., Sadras, V.O., Uhart, S.A., Valentinuz, O.R., 2001. Seed number as a function of growth. A comparative study in soybean, sunflower, and maize. Crop Sci. 41, 748–754.
Vega, C.R.C., Sadras, V.O., Andrade, F.H., Uhart, S.A., 2000. Reproductive allometry in soybean, maize and sunflower. Ann. Bot. 85, 461–468.