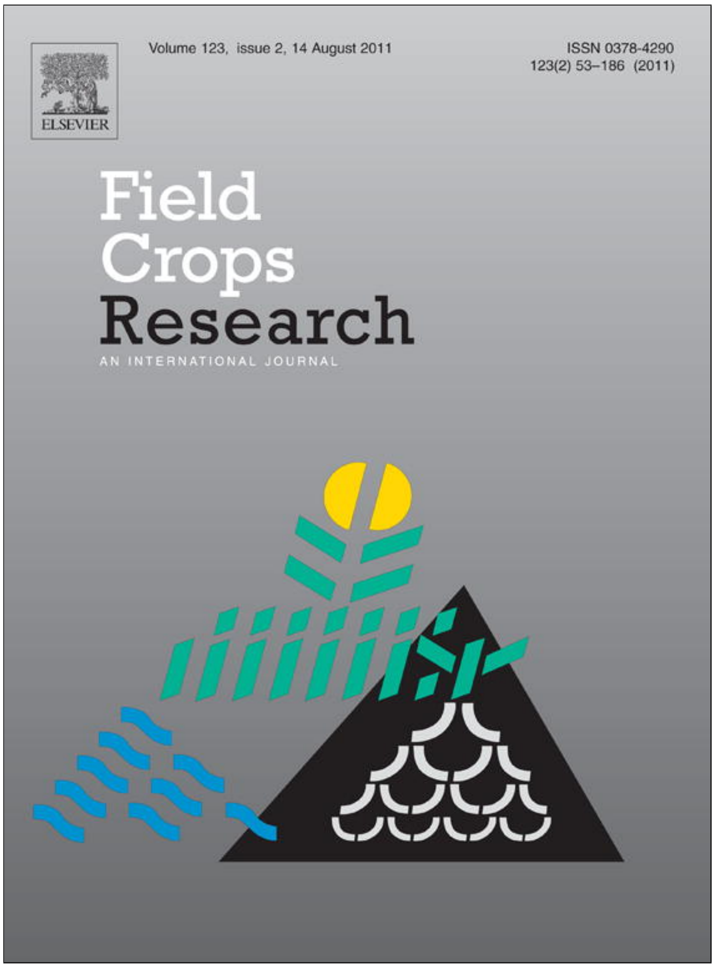
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Heat stress effects around flowering on kernel set of temperate and tropical maize hybrids

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ABSTRACT

Final kernel number in the uppermost ear of temperate maize (*Zea mays* L.) hybrids is smaller than the potential represented by the number of florets differentiated in this ear, and than the number of silks exposed from it (i.e., kernel set <1). This trend increases when stressful conditions affect plant growth immediately before (GS_1) or during (GS_2) silking, but the magnitude of change has not been documented for heat stress effects and hybrids of tropical background. In this work we evaluated mentioned traits in field experiments (Exp_1 and Exp_2), including (i) two temperature regimes, control and heated during daytime hours (ca. 33–40 °C at ear level), (ii) two 15-d periods during GS_1 and GS_2, and (iii) three hybrids (Te: temperate; Tr: tropical; TeTr: Te × Tr). We also measured crop anthesis and silking dynamics, silk exposure of individual plants, and the anthesis–silking interval (ASI). Three sources of kernel loss were identified: decreased floret differentiation, pollination failure, and kernel abortion. Heating affected all surveyed traits, but negative effects on flowering dynamics were larger (i) for anthesis than for silking with the concomitant decrease in ASI, and (ii) for GS_1 than for GS_2. Heat also caused a decrease in the number of (i) florets only when performed during GS_1 (−15.5% in Exp_1 and −9.1% in Exp_2), and only among Te and TeTr hybrids, (ii) exposed silks of all GS × Hybrid combinations, and (iii) harvestable kernels (mean of −51.8% in GS_1 and −74.5% in GS_2). Kernel abortion explained 95% of the variation in final kernel numbers ($P < 0.001$), and negative heat effects were larger on this loss (38.6%) than on other losses (≤11.3%). The tropical genetic background conferred an enhanced capacity for enduring most negative effects of heating.

1. Introduction

Final kernel number of grain crops is the result of successive steps that start with reproductive initiation in specific meristems (Bonnett, 1966). In maize (*Zea mays* L.), these steps take place simultaneously in several axillary buds along the stem, but usually only two of them (apical and subapical) reach successful kernel set (i.e., kernel per developed floret). The developmental events experienced by these buds have been thoroughly analyzed from the botanical point of view (Bonnett, 1966; Ruget and Duburcq, 1983; Stevens et al., 1986). Additional information was produced regarding the response of floret development (i.e., determination of potential kernel numbers) to breeding effects (Edmeades et al., 1993), and to variation in agronomic practices like sowing date (Cirilo and Andrade, 1994b; Otegui and Melón, 1997) and stand density (Otegui, 1997). We also know about (i) the exact pattern of silk emergence from different positions along the ear (Bassetti and Westgate, 1993a), (ii) the persistence of silk viability (Bassetti and Westgate, 1993a,b), and (iii) the effect of stand density on the dynamics of silk emergence from the ear (Cárcova et al., 2000; Uribelarrea et al., 2002). Most part of this knowledge has been reviewed (Otegui and Andrade, 2000; Westgate et al., 2004), and carefully summarized in simulation models for the estimation of final kernel number in this species (Lizaso et al., 2003; Fonseca et al., 2004).

Abbreviations: ASI, anthesis–silking interval; CST, cumulative stressful temperatures; E_1, apical ear; Exp_n, experiment n; GS_n, growth stage n; H, hybrid; KNE, kernel number per E_1; KSE_1, kernel set per developed floret in E_1; KSE_2, kernel set per exposed silk in E_1; NES, number of exposed silks from E_1; FPE, florets number per E_1; Pop, proportion of the population of plants; T_C, non-heated control plot; Te, temperate hybrid; TeTr, temperate per tropical hybrid; T_H, heated plot; T_{max}, maximum temperature; Tr, tropical hybrid; TR, temperature regime.

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The abundant information described above, however, is yet incomplete. Most research has been limited to germplasm of temperate origin. Only a few reports addressed some aspects of flowering dynamics (e.g., duration of the anthesis–silking interval) and kernel set (e.g., relationship between final kernel number and total ovule number) in genotypes of tropical genetic background (Fischer and Palmer, 1984; Edmeades et al., 1993; Monneveux et al., 2005, 2006). No one is complete respect to the quantification of all quantitative determinants of final kernel set (Otegui and Andrade, 2000); e.g., they lack information on the total number of exposed silks.

As for genotypes, interest on mentioned determinants focused on potential growing conditions (Otegui and Andrade, 2000; Westgate et al., 2004) or addressed some limitations produced by water or N stress (Bassetti and Westgate, 1993c; Edmeades et al., 1993). Studies on abiotic stress effects (i) never surveyed the response of all determinants (i.e., number of florets, silks exposed, and kernel set), and (ii) there is no reference of their variation in response to the occurrence of heat stress around flowering, which is a frequent event in tropical environments (Lobell et al., 2011).

In a recent research (Cicchino et al., 2010a) on the response of a temperate hybrid to heat stress imposed during the late-vegetative period (i.e., during 15 days immediately before anthesis), authors registered the expected delayed in flowering events (i.e., mean dates of anthesis and silking). Interestingly, they did not detect the pronounced increase in the anthesis–silking interval (ASI) usually reported when this type of germplasm is subjected to other abiotic constraints (Hall et al., 1982; Jacobs and Pearson, 1991). This was attributed to the fact that heat did not reduce biomass partitioning to the ear (Cicchino et al., 2010b) as observed for water (Echarte and Tollenaar, 2006) and nitrogen (D'Andrea et al., 2008) deficiencies. There were severe effects on final kernel number due to reduced overall biomass production under high temperature regimes, but authors gave no information on the relative effects of heating on potential ear size and final number of silks exposed to pollen.

Finally, most research on the pattern of silk emergence of individual plants is based on countings performed on bagged ears (Bassetti and Westgate, 1993a; Cárcova et al., 2000; Lizaso et al., 2003), a technique that may introduce a bias respect to the actual dynamics of natural pollinated individuals. Differences in ovary fresh weight evolution (Cárcova and Otegui, 2007) and final kernel set (Cárcova et al., 2000; Cárcova and Otegui, 2001) between natural and bagged ears (i.e., those used for artificial manipulation of pollination) indicated that late-pollinated ovaries from the tip of this organ experienced an interference exerted by the early-pollinated ones from the base. This interference was also evident in relative silk growth along the ear (Cárcova et al., 2003), and may have consequences on the final number of exposed silks. Therefore, correct quantification of this trait in ordinary production conditions requires evaluation of natural pollinated plants rather than of non-pollinated individuals.

In the current research we analyzed the variation in potential floret number, total number of exposed silks and final kernel number of three F1 maize hybrids of different genetic background (temperate, tropical and temperate × tropical) subjected to natural pollination. We evaluated the response of mentioned traits when these hybrids were grown under two contrasting temperature regimes around silking: normal ambient temperature and above-optimum temperature (Ritchie and NeSmith, 1991). Data were used for the computation of kernel set per floret and per exposed silk for each treatment combination (Cárcova et al., 2000), but also for the evaluation of different ways of loss in potential kernel number represented by the maximum number of florets per ear of each hybrid.

2. Materials and methods

2.1. Crop husbandry and experimental design

Field experiments were conducted during 2008–2009 (Exp_1) and 2009–2010 (Exp_2) at the experimental field of the University of Buenos Aires (34°25′S, 58°25′W), on a silty clay loam soil (Vertic Argiudol). Treatments included a factorial combination of three F1 hybrids of contrasting genetic background (Te: temperate, Tr: tropical, and TeTr: temperate × tropical), and two temperature regimes (T_C: control with no heating; T_H: heated during daytime hours) applied during two different growth stages around flowering (GS_1: 15 days before anthesis; GS_2: 15 days from start of silking onwards). Hybrids (H) were 2M545 HX (Te), 2B710 HX (Tr), and 2A120 HX (TeTr). All hybrids were produced by Dow Agrosciences Argentina, and recommended for different environments: (i) Te for the central temperate region of Argentina (above 30°S; 58–65°W), (ii) Tr for the northwest subtropical region of the country (22–28°S; 62–66°W), and (iii) TeTr for all the transition area between the temperate and subtropical regions of the country (below 30°S; 53.7–66°W). Inbreds used for producing each of these hybrids share common heterotic backgrounds and have no significant response to photoperiod (S. Uhart, Dow Agrosciences, pers. comm.). Sowing started late (December) and took place at different dates for each H × GS combination (Table 1). This was done for ensuring (i) the achievement of differential temperature regimes (TR) after the summer period of highest irradiance and temperature, in order to avoid overheating of T_H plots, and (ii) the simultaneous occurrence of all H × GS combinations (Fig. 1). This concurrence was necessary in order to avoid the confounded effect of the environment (i.e., natural decay of irradiance and temperature after the summer solstice) on treatment evaluation because of the wide range of relative maturities (RM) among tested hybrids (RM Te = 124; RM TeTr = 128; RM Tr = 136). Experiments were hand-planted at three seeds per hill, and thinned to the desired plant population at the three-liguIated leaf stage (V_3; Ritchie et al., 2008). A single stand density of 9 plants m^{-2} was used. The experimental site was fertilized with 200 kg N ha^{-1} at V_6. P and K were not added because high levels of both elements were present in the experimental site due to their addition in previous experiments. Pests, weeds and diseases were adequately controlled. Water availability of the uppermost 1 m of soil was kept near field capacity throughout the growing season by means of drip irrigation.

Treatments were distributed in a split split-plot design, with GS_n in main plots, hybrids in subplots and temperature regimes in sub-subplots (hereafter termed plots). Three replicates were always used. Plots were 10 m length, with six rows separated at 0.5 m between rows. Temperature treatments covered 3 m along the four central rows (6 m^2). These treatment areas were enclosed with polyethylene film (100-μm thickness) fixed to wood stalks (laterals and top), yielding rigid shelters of 3.5 m height (see Cicchino et al., 2010a). For avoiding the accumulation of rainfall water on the roof, a parabolic shape was established by means of plastic tubes fixed to the wood structure. Additionally, roofs of all shelters were pierced for avoiding excessive heating at the top of the canopy, which also helped gas exchange. One shelter was for T_H and had the film reaching the soil surface on all sides, except one side that had a 10-cm opening at the bottom for allowing adequate gas exchange. The other shelter was for T_C and had laterals open up to 1.4 m above soil surface. Open shelters were used for avoiding differences in light offer due to polyethylene film. Heating of T_H treatments depended mainly on temperature rise promoted by the greenhouse effect of polyethylene enclosure (Cicchino et al., 2010a). Nonetheless, it was supplemented by an equipment made of a portable electric fan heater connected to a temperature sensor (TC1047, Microchip Technologies, Chandler, AZ), all monitored by

Table 1
Detail of experiments.

Experiment	Growth stage	Hybrid	Sowing date	T_{max} (°C)[a]	CST (°C h)
Exp_1	GS_1	Te	22-Dec-08	36.1 ± 0.1[b]	144 ± 36
		TeTr	22-Dec-08	37.4 ± 0.2	210 ± 63
		Tr	16-Dec-08	34.0 ± 0.2	71 ± 35
	GS_2	Te	9-Dec-08	36.6 ± 0.8	236 ± 52
		TeTr	9-Dec-08	36.6 ± 1.6	228 ± 125
		Tr	2-Dec-08	35.8 ± 0.9	190 ± 67
Exp_2	GS_1	Te	18-Dec-09	35.6 ± 0.5	107 ± 21
		TeTr	18-Dec-09	36.2 ± 1.7	107 ± 49
		Tr	11-Dec-09	35.9 ± 0.4	146 ± 119
	GS_2	Te	3-Dec-09	33.6 ± 4.0	111 ± 30
		TeTr	3-Dec-09	34.9 ± 2.0	145 ± 53
		Tr	20-Nov-09	35.5 ± 1.8	129 ± 81
Source of variation					
Exp				ns	ns
GS				ns	0.0014[c]
H				ns	ns
Exp × GS				0.002	0.003
Exp × H				ns	ns
GS × H				ns	ns
Exp × GS × H				ns	ns

[a] T_{max}: mean maximum temperature during treatment period. CST: cumulative stressful temperatures; Exp: experiment; GS: growth stage; Te: temperate; Tr: tropical; TeTr: Te × Tr; H: Hybrid.
[b] Mean ± SD.
[c] P values of main and interaction effects; ns: not significant ($P > 0.05$).

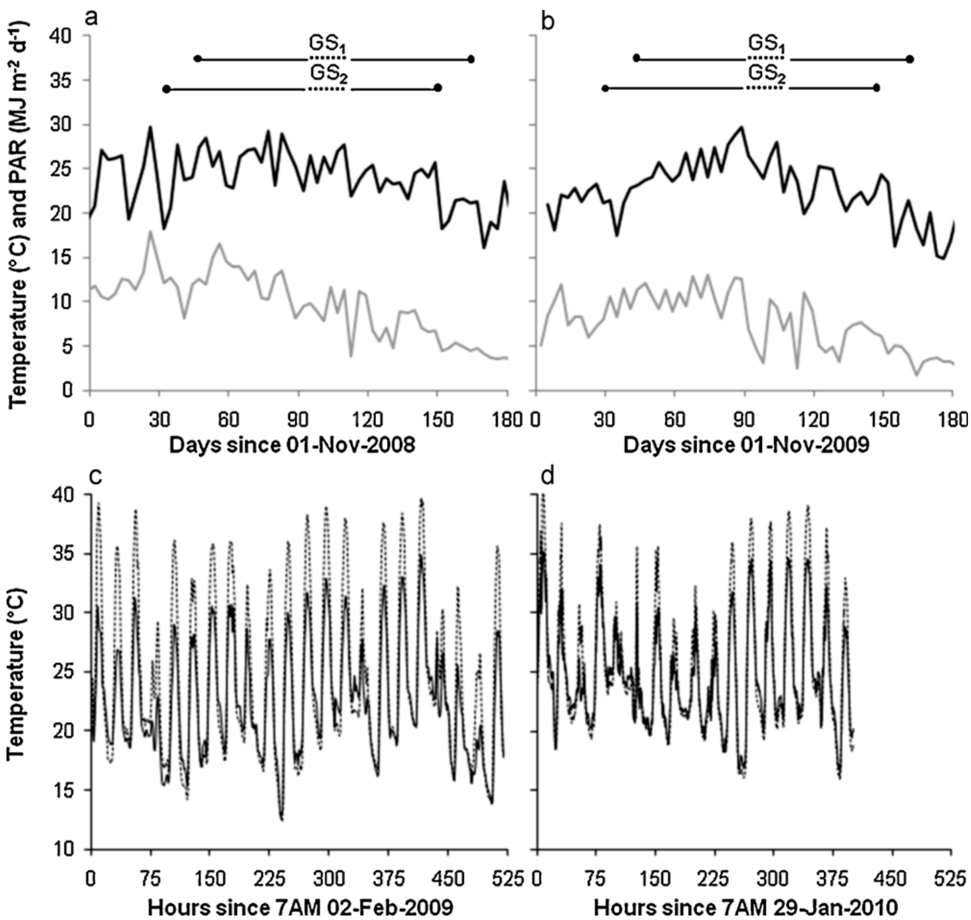

Fig. 1. Mean daily air temperature (black line) and solar radiation (grey line) evolution during the crop cycle (uppermost figures), and average hourly air temperature evolution at ear height of non-heated (black line) and heated plots (grey line) during the treatment period (lowermost figures). In (a) and (b), the solid horizontal line represents the time from emergence to physiological maturity and the dashed bit represents the treatment periods, both averaged across hybrids. Data correspond to two growing seasons: 2008–2009 (a and c), and 2009–2010 (b and d). GS_1 represents the preanthesis treatment (ca. 15 days immediately before anthesis) and GS_2 the silking treatment (ca. 15 days starting at the beginning of silking of the population of plants).

an automated control unit (Cavadevices, Buenos Aires, Argentina). The system was programmed for (i) starting heating at 800 h, (ii) producing a gradual increase in temperature until a maximum of 40 °C was reached at ear level at 1200 h, and (iii) holding temperature close to this maximum for four hours. The heater stopped each time the sensor detected 40 °C, but the fan was permanently operating during the heated period for reducing temperature variation at different positions within the shelter. Heating of GS_1 started when 50% of the plants in T_C plots of each hybrid reached ca. V_{15}–V_{17} and finished when 10% of these plants reached anthesis. For GS_2, the heating period extended between the beginning of silking (ca. 10%) of plants in T_C plots of each hybrid and finished 15 days later. All shelters were removed at the end of each heating period.

2.2. Measurements, computations and statistical analyses

Daily incident photosynthetically active radiation (PAR, in $MJ\,m^{-2}\,d^{-1}$) and mean air temperature were registered at the experimental site (Weather Monitor II, Davis Instruments, USA). Additionally, air temperature of each shelter (T_H and T_C) was recorded hourly throughout the treatment period by means of a sensor (independent of the one described for the heating unit) connected to a datalogger (Temp-Logger, Cavadevices, Buenos Aires, Argentina). These sensors were shielded in double-walled plastic cylinders with open ends, which were positioned in the center of each plot at the uppermost ear level (Cicchino et al., 2010a). Additional sensors were placed at the top of the canopy to monitor air temperature at this level (data not shown), in order to avoid temperature rise above 50 °C (Monteith and Unsworth, 1990). Heat stress was computed for each plot as cumulative stressful temperatures (CST, in °C h; Eq. (1)):

$$\mathrm{CST} = \sum_{i=1}^{N} (T_X - T_O) \quad (1)$$

where N is the duration of treatment period (in hours), T_X is air temperature (in °C), and T_O is optimum temperature (in °C). T_O was estimated for each hybrid by means of the algorithm developed by Cicchino et al. (2010a). It was set always at 33 °C because no significant difference was detected among them, in agreement with previous findings on genetic variation of cardinal temperatures in maize (Ritchie and NeSmith, 1991; Padilla and Otegui, 2005).

Forty-six plants were tagged within each sheltered area at V_{11}. The dates of anthesis (i.e., at least one extruded anther visible) and silking (i.e., at least one extruded silk visible) were recorded on all tagged plants. The progress of each stage was described using a sigmoid logistic function (Eq. (2)) fitted to the whole data set of each flowering event (Lizaso et al., 2003):

$$\mathrm{Pop} = \frac{a}{\{1 + \exp[(-(X - b)/c)]\}} \quad (2)$$

where Pop is the proportion of plant population that reached the stage, a is the maximum proportion of plant population that reached the stage, b is time to 50% of the value represented by parameter a (in days), and c is a parameter governing maximum slope (in days). If maximum observed Pop = 1, then a = 1 and Eq. (2) had only two estimated parameters (b and c). The ASI of the population of plants (ASI_{PP}) was calculated for each plot as the difference in days between 50% silking and 50% anthesis dates. For comparison among treatments, all data (i.e., anthesis and silking) were standardized to the start of anthesis of the corresponding T_C plot of each GS × H combination, and day 0 was set on the day before the first tagged plant reached anthesis.

Adequate pollination and fertilization of all plants was granted in the experiments. For T_H plots, fresh pollen was collected daily from non-heated plants (i.e., from the same experiment and from additional plots sown later than the experimental plots) and was added manually to silks exposed from all silked ears of tagged plants. Silks were pollinated by hand between 900 and 1100 h. Pollination continued until no new silks were exposed from among the husks, and the arrest of silk elongation 24 h after pollination was evidence of a successful procedure (Bassetti and Westgate, 1993a,b).

Three tagged plants of each shelter were used for silk counting (only in Exp_2). These plants were selected from different percentiles of the silking population of plants (early silking 25%, mean 50% and late silking 75%), in order to include all the expected variation among individuals (Borrás et al., 2007, 2009; Pagano et al., 2007). Exposed sections of silks were cut from the apical ear (E_1) of these plants on 1 (day 2), 3 (day 4), and 5 (day 6) days after first silks were visible (day 1). All newly exposed silks (i.e., those with a bisected hairy end) were counted to develop a cumulative curve of silk emergence (Cárcova et al., 2000). Day 0 was set on the day before first silks were exposed from E_1 of each tagged plant. The total number of exposed silks per E_1 (NES) was calculated as the cumulative amount of newly exposed silks on day 6. Within each GS × H combination, the number of silks exposed on each date from all plant categories and temperature regimes was referred to the maximum number registered on day 6, which usually corresponded to early silking plants of T_C plots. Ears sampled on day 6 (three per plot) were harvested on day 7 for counting total floret number in E_1 (FPE). In both experiments, ten additional tagged plants were used for counting FPE and were collected between R_3 and mid grain filling. In all these ears, the number of completely developed flowers (i.e., those with a visible silk of at least 1 mm; Cárcova et al., 2000) was counted on two opposite rows of spikelets along the ear, and the average value was multiplied by the total number of rows for obtaining FPE. During GS_2 of Exp_1, this trait was measured only on ears collected from T_C plots of each hybrid and assumed as representative of all temperature regimes, because floret differentiation arrests at (Ruget and Duburcq, 1983; Fischer and Palmer, 1984) or immediately before silking (Otegui, 1997; Otegui and Melón, 1997). It was measured in all plots during Exp_2.

Kernel number per apical ear (KNE) was counted on the remaining tagged plants at physiological maturity. Kernel set per apical ear (KSE) was obtained as the quotient between (i) KNE and FPE (KSE_1), and (ii) KNE and NES (KSE_2). The number of grained ears per plant (i.e., prolificacy) was also computed at this stage. All ears having at least one grain were considered fertile.

Three sources of loss were established between the potential kernel number (i.e., FPE) and the actual kernel number (i.e., KNE). The first loss (Loss 1) represented the decrease in the number of potential florets (i.e., morphogenetic restriction at the axillary meristem level). It was null for T_C plots (Loss 1 T_C = 0) and computed as in Eq. (3) for T_H plots.

$$\mathrm{Loss}\ 1 \cdot T_H = \frac{(\mathrm{FPE} \cdot T_C - \mathrm{FPE} \cdot T_H)}{\mathrm{FPE} \cdot T_C} \quad (3)$$

The second loss (Loss 2) represented the proportion of florets that did not reach silking (i.e., pollination failure), and was computed for each treatment combination as in Eq. (4):

$$\mathrm{Loss}\ 2 = 1 - \left(\frac{\mathrm{NES}}{\mathrm{FPE}}\right) \quad (4)$$

The effect of heating on this source of loss was established as the difference between values obtained for heated (Loss 2 T_H) and non-heated plots (Loss 2 T_C).

The third loss (Loss 3) represented the proportion of pollinated silks that did not produce a harvestable kernel. Because fresh, non-heated pollen was spreaded daily on silks of each tagged plant along silking, this loss was assumed as representative of kernel abortion

Table 2
Descriptors of flowering dynamics.

Exp[a]	GS	H	TR	Anthesis			Silking			ASI_{PP} (days)
				a	*b* (days)	*c* (days)	*a*	*b* (days)	*c* (days)	
Exp_1	GS_1	Te	T_C	1.00	2.85	0.70	0.99	3.78	0.77	−0.33
			T_H	0.64	12.38	1.45	0.82	7.21	1.50	−8.00
		TeTr	T_C	0.99	2.56	0.61	0.97	2.64	0.65	−1.00
			T_H	0.97	10.23	1.18	0.78	6.04	2.21	−3.00
		Tr	T_C	0.99	3.35	0.60	1.00	3.96	0.76	−0.67
			T_H	0.68	9.10	0.94	0.99	5.61	1.20	−7.00
	GS_2	Te	T_C	1.00	3.26	0.87	1.00	4.95	1.59	1.33
			T_H	0.71	5.11	2.21	0.83	5.81	1.06	1.50
		TeTr	T_C	1.00	4.05	0.72	1.00	4.57	1.21	0.67
			T_H	0.88	3.55	0.63	0.89	4.36	1.11	1.00
		Tr	T_C	0.99	2.84	1.06	0.98	5.21	1.05	3.00
			T_H	0.98	3.15	1.01	0.92	6.94	1.18	2.00
Exp_2	GS_1	Te	T_C	1.00	3.50	0.78	1.00	2.47	1.27	−1.00
			T_H	(0.00)	–	–	0.85	6.68	1.04	–
		TeTr	T_C	0.95	3.12	0.43	0.99	3.07	1.29	0.33
			T_H	(0.18)	–	–	0.91	7.11	1.38	–
		Tr	T_C	1.00	3.27	0.68	0.98	4.38	0.98	1.00
			T_H	(0.04)	–	–	1.00	8.85	1.28	–
	GS_2	Te	T_C	1.00	3.92	1.00	0.97	4.58	1.11	1.00
			T_H	0.86	4.37	0.76	0.89	5.64	1.16	1.00
		TeTr	T_C	0.99	1.86	0.47	0.96	1.86	0.60	0.00
			T_H	0.82	1.89	0.63	0.93	1.45	0.53	−1.00
		Tr	T_C	0.96	2.96	0.72	0.96	3.99	0.89	1.67
			T_H	0.94	4.00	0.71	0.91	4.43	1.55	1.67

Source of variation	All Exp	Exp_1	Exp_2	Exp_1	Exp_2	All Exp	All Exp	All Exp	Exp_1	Exp_2
Exp	0.008[b]	–	–	–	–	ns	ns	ns	–	–
GS	0.001	0.002	–	ns	–	ns	ns	ns	0.009	–
H	ns	ns	ns	ns	ns	ns	0.007	ns	ns	ns
TR	<0.001	<0.001	ns	0.040	ns	<0.001	<0.001	0.002	0.002	ns
Exp × GS	0.003	–	–	–	–	ns	0.015	ns	–	–
Exp × TR	<0.001	–	–	–	–	ns	ns	ns	–	–
GS × H	ns	ns	–	ns	–	ns	ns	0.010	ns	–
GS × TR	<0.001	<0.001	–	0.002	–	ns	<0.001	0.004	0.003	–
H × TR	ns	ns	ns	ns	ns	0.011	ns	ns	ns	ns
Exp × GS × H	ns	–	–	–	–	ns	0.031	ns	–	–
Exp × GS × TR	<0.001	–	–	–	–	ns	ns	<0.001	–	–

[a] Exp: experiment; GS: growth stage; H: Hybrid; TR: temperature regime. *a*: maximum proportion of plant population that reach the event; *b*: time to 50% of the value represented by parameter *a*. *c*: parameter governing maximum slope. ASI_{PP}: anthesis–silking interval of the population of plants; 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 ($P > 0.05$).

in the apical ear (Westgate and Boyer, 1986a; Otegui et al., 1995a) and computed as in Eq. (5):

$$\text{Loss 3} = 1 - \left(\frac{\text{KNE}}{\text{NES}}\right) \quad (5)$$

As computed for Loss 2, the effect of heating on Loss 3 was established as the difference between values obtained for heated (Loss 3 T_H) and non-heated plots (Loss 3 T_C).

The absolute loss was computed as in Eq. (6):

$$\text{absolute loss} = 1 - \left(\frac{\text{KNE}}{\text{FPE} \cdot \text{T}_\text{C}}\right) \quad (6)$$

where KNE corresponds to each treatment combination (i.e., GS × H × TR) and FPE corresponds to T_C plots for each GS × H combination (i.e., actual potential number). Heat effects were estimated as the difference between values computed for heated (absolute loss T_H) and control plots (absolute loss T_C).

All data were analyzed by ANOVA to evaluate the effects of treatments and their interactions, each based on the corresponding source of error of a split split-plot design. A *t*-test was used to determine significant differences ($P < 0.05$) between means. The relationship between variables was analyzed by linear regression.

3. Results

3.1. Growing conditions

Experimental years exposed the crops to very contrasting growing conditions due to the occurrence of La Niña (2008–2009) and El Niño (2009–2010) phases of the El Niño Southern Oscillation (ENSO) phenomenon (Anonymous, 2010). Consequently, the treatment period was characterized by sunny days in Exp_1 (mean PAR values of 8.8 MJ m^{-2} d^{-1}) and by cloudy skies in Exp_2 (mean PAR values of 7.4 MJ m^{-2} d^{-1}). However, mean air temperature during this period was slightly higher during Exp_2 (24.6 °C) than during Exp_1 (22.4 °C). In spite of this situation, spaced sowings allowed the almost simultaneous occurrence of all GS × H combinations within each experiment (Fig. 1a and b). The time elapsed between the installation and removal of the first and last heating shelters, respectively, was 22 days in Exp_1 (Fig. 1c) and 17 days in Exp_2 (Fig. 1d).

Heating increased air temperature at ear level during treatment period (Fig. 1c and d). Differences in this variable between T_H and T_C 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). During the same period, daily absolute maximum air temperature (T_{max}) at ear height of T_H plots increased between 1.3 °C and 8.7 °C

Table 3
Determinants of final kernel numbers, kernel set and kernel loss.

Exp[a]	GS	H	TR	FPE	NES	NES FPE^{-1}	Prolificacy (ears pl^{-1})	KNE	KSE_1	KSE_2	Absolute loss	Heat effect
Exp_1	GS_1	Te	T_C	682	–	–	0.89	351	0.52	–	0.49	
			T_H	570	–	–	0.78	140	0.25	–	0.80	0.31
		TeTr	T_C	670	–	–	1.00	320	0.48	–	0.52	
			T_H	522	–	–	0.85	125	0.24	–	0.81	0.29
		Tr	T_C	687	–	–	0.96	334	0.49	–	0.51	
			T_H	632	–	–	1.00	339	0.54	–	0.51	−0.01
	GS_2	Te	T_C	780	–	–	0.89	337	0.43	–	0.57	
			T_H	780[b]	–	–	0.19	23	0.03	–	0.97	0.40
		TeTr	T_C	635	–	–	1.00	322	0.51	–	0.49	
			T_H	635	–	–	0.67	130	0.21	–	0.80	0.30
		Tr	T_C	736	–	–	0.96	392	0.53	–	0.47	
			T_H	736	–	–	0.85	183	0.25	–	0.75	0.28
Exp_2	GS_1	Te	T_C	668	515	0.77	1.00	392	0.59	0.76	0.41	
			T_H	599	404	0.67	0.67	108	0.18	0.27	0.84	0.43
		TeTr	T_C	627	451	0.72	0.96	375	0.60	0.84	0.40	
			T_H	522	380	0.73	0.85	144	0.28	0.38	0.77	0.37
		Tr	T_C	715	591	0.83	1.00	464	0.65	0.79	0.35	
			T_H	708	495	0.70	0.93	200	0.29	0.42	0.72	0.37
	GS_2	Te	T_C	727	581	0.80	0.93	213	0.30	0.37	0.71	
			T_H	678	452	0.67	0.22	39	0.06	0.10	0.95	0.24
		TeTr	T_C	650	529	0.82	1.00	234	0.36	0.42	0.64	
			T_H	710	438	0.62	0.19	13	0.02	0.03	0.98	0.34
		Tr	T_C	723	571	0.79	1.00	283	0.39	0.49	0.61	
			T_H	722	467	0.65	0.70	93	0.13	0.20	0.87	0.26

Source of variation	Exp_1	Exp_2	Exp_2	Exp_2	All Exp	All Exp	All Exp	Exp_2	All Exp	All Exp
Exp	–	–	–	–	ns	ns	ns	–	ns	ns
GS	–	ns	0.046[c]	ns	0.014	0.004	0.002	0.001	0.003	ns
H	ns	0.015	ns	ns	<0.001	0.002	0.004	ns	0.013	ns
TR	<0.001	0.011	<0.001	0.005	<0.001	<0.001	<0.001	<0.001	<0.001	–
Exp × GS	–	–	–	–	ns	0.026	0.034	–	0.031	0.012
Exp × TR	–	–	–	–	0.021	ns	ns	–	ns	–
GS × H	–	ns	ns	ns	0.021	ns	ns	ns	ns	ns
GS × TR	–	0.005	ns	ns	<0.001	ns	ns	ns	ns	–
H × TR	ns	ns	ns	ns	0.001	ns	ns	ns	ns	–
Exp × GS × TR	–	–	–	–	ns	0.012	0.014	–	0.009	–
GS × H × TR	–	0.008	ns	ns	ns	ns	ns	ns	ns	–

[a] Exp: experiment; GS: growth stage; H: Hybrid; TR: temperature regime; FPE: florets per apical ear; NES: silks exposed from apical ear; KNE: kernel number per apical ear; KSE_1: KNE FPE^{-1}; KSE_2: KNE NES^{-1}. Absolute loss: failure to set a kernel respect to reference FPE (i.e., that of T_C plots for each GS × H combination). Heat effect: proportion of absolute loss that can be attributed exclusively to heat effects, computed as the difference between T_H and T_C plots. Te: temperate; Tr: tropical; TeTr: Te × Tr; T_C: non-heated control; T_H: heated.

[b] No distinction between T_H and T_C plots during GS_2 of Exp_1 (i.e., only one value of FPE for each hybrid).

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

as compared to their non-heated counterparts, depending upon the variation in daily incident PAR ($T_{max} = 29.38 + 0.70$ PAR, $r^2 = 0.58$, $P < 0.001$). Within each experiment, the intensity of heat stress was similar for each GS × H combination (Table 1), but large differences were computed between experiments. In spite of the similar value obtained for mean T_{max} (mean of daily T_{max} records during treatment period) of T_H plots (36 °C in Exp_1 and 35.3 °C in Exp_2), the intensity of stress was larger for Exp_1 (average CST of 180 °C h, Table 1) than for Exp_2 (average CST of 120 °C h, Table 1).

3.2. Flowering dynamics

Heat stress always affected flowering dynamics, and caused significant differences ($P < 0.05$) between temperature regimes in the parameters of fitted sigmoid curves (Table 2). In general, these differences were larger for GS_1 than for GS_2. All plants reached tasseling (VT), but heating during the late-vegetative period (GS_1) was accompanied by (i) a decline in the proportion of plants that reached anthesis and silking (i.e., reduced value of parameter a in Eq. (2)), (ii) a delay in the mean date of both flowering events (i.e., enhanced value of parameter b in Eq. (2)), and (iii) a reduction in the rate of these events (i.e., enhanced value of parameter c in Eq. (2); significant only in Exp_1). Heat stress during GS_2 had a negative effect only on parameter a.

The proportion of plants that reached anthesis or silking was reduced ($P < 0.005$) in all heated plots, but the effect was larger on the former than on the latter (Table 2). There was a clear effect of heat stress on flowering of the male organ, evident as tassels with no or few extruded anthers (visual assessment). The proportion of plants that reached anthesis under heat stress was similar between treatment periods of Exp_1 (0.76 in GS_1 and 0.86 in GS_2; averaged across hybrids), but differed markedly during Exp_2 (0.07 in GS_1 and 0.87 in GS_2). The proportion of heated plants that reached silking did not differ between treatment periods at any experiment (0.86 in GS_1 and 0.89 in GS_2 of Exp_1; 0.92 in GS_1 and 0.91 in GS_2 of Exp_2).

All flowering events were delayed by heating (parameter b) at any GS. Almost complete lack of anthesis among tagged plants of all hybrids heated during GS_1 in Exp_2 (≤18%, Table 2) did not allow for adequate fit of Eq. (2), and hindered statistical comparisons for this trait between temperature regimes in this condition. Because of this constraint, the analysis of anthesis revealed significant heat effects only for GS_1 in Exp_1 (Table 2). In this growing condition, it caused a difference of 7.6 days between parameters b obtained for T_C and T_H plots (averaged across hybrids). This difference increased to 8.4 days when the computation was based on 50% anthesis under each temperature regime (data not shown). Same analysis of the silking event revealed a difference between T_C and T_H plots (averaged across hybrids and experiments) of (i) 2.8 (GS_1) or 0.8 days (GS_2)

Table 4
Sources of loss between potential and final kernel numbers.

GS[a]	H	TR	Experiment 1		Experiment 2					
			Loss 1	Heat effect[b]	Loss 1	Heat effect	Loss 2	Heat effect	Loss 3	Heat effect
GS_1	Te	T_C	0		0		0.23		0.24	
		T_H	0.16	0.16	0.10	0.10	0.33	0.10	0.73	0.49
	TeTr	T_C	0		0		0.28		0.17	
		T_H	0.22	0.22	0.17	0.17	0.27	−0.01	0.62	0.45
	Tr	T_C	0		0		0.17		0.22	
		T_H	0.08	0.08	0.01	0.01	0.30	0.13	0.60	0.38
GS_2	Te	T_C	–		0		0.20		0.63	
		T_H	–	–	0.07	0.07	0.33	0.13	0.91	0.28
	TeTr	T_C	–		0		0.19		0.56	
		T_H	–	–	−0.09	−0.09	0.38	0.19	0.97	0.41
	Tr	T_C	–		0		0.21		0.50	
		T_H	–	–	0.00	0.00	0.35	0.14	0.80	0.30
Source of variation										
GS			–	–	0.009[c]	0.002	ns	0.05	0.01	ns
H			–	ns	ns	ns	ns	ns	ns	ns
TR			<0.001	–	0.007	–	0.005	–	<0.001	–
GS × H			–	–	0.02	0.02	ns	ns	ns	ns
GS × TR			–	–	0.002	–	ns	–	ns	–
H × TR			ns	–	ns	–	ns	–	ns	–
GS × H × TR			–	–	0.003	–	ns	–	ns	–

[a] GS: growth stage; H: Hybrid; TR: temperature regime; Te: temperate; Tr: tropical; TeTr: Te × Tr; T_C: non-heated control; T_H: heated. Loss 1: due to reduced florets per ear. Loss 2: due to floret failure to expose a silk. Loss 3: due to kernel abortion.
[b] Heat effects represent the difference in each source of loss between T_H and T_C plots.
[c] P values of main and interaction effects. ns: not significant ($P > 0.05$).

when based on parameter b, and (ii) 4.1 (GS_1) or 0.5 days (GS_2) when based on 50% of plant population.

Described trends of the effects of heating on flowering events caused significant ($P < 0.01$) reductions in the ASI_{PP} of plots heated during GS_1 in Exp_1 (−5.3 days). This reduction could not be assessed statistically for Exp_2 due to mentioned lack of anthesis in many plots. Contrasting temperature regimes during GS_2 did not modify the ASI_{PP} significantly.

Heat stress reduced ($P < 0.05$) the rate of all flowering events during Exp_1 (i.e., enhanced values of parameter c, Table 2). However, a significant ($P < 0.01$) GS × TR interaction effect was detected for both events. This trend identified GS_1 as the only period when contrasting temperature regimes modified flowering rates ($T_H < T_C$). Computed c values for GS_1 in Exp_1 (averaged across hybrids) ranged between (i) 1.19 (T_H) and 0.64 (T_C) for anthesis, and (ii) 1.64 (T_H) and 0.73 (T_C) for silking.

3.3. Potential ear size and pattern of silk emergence

Heat stress caused a decrease in potential ear size (FPE; $P < 0.01$, Table 3) only when it was performed during GS_1 (−15.5% in Exp_1 and −9.1% in Exp_2). The significant GS × H × TR interaction ($P = 0.008$, Table 3) detected for this trait during Exp_2 was due to the reduction observed in heated plots of the TeTr (−16.5%) and the Te (−10.2%) hybrids only during GS_1. This trend was not registered for the Tr hybrid (Table 3). Mentioned reductions in FPE caused a loss in final kernel numbers (Loss 1, Table 4), for which a significant ($P \leq 0.007$) proportion could be attributed to the temperature regime (Loss1 T_H > Loss 1 T_C). Additionally, the significant ($P = 0.003$) GS × H × TR interaction detected during Exp_2 indicated that the largest magnitude registered for this loss corresponded to heated plots of TeTr (17%) and Te (10%) hybrids during GS_1, with almost no effect on other treatment combinations (Table 4).

Treatments affected the number of exposed silks (NES, Table 3). Mean maximum values (day 6) corresponded to the Tr hybrid (Tr ≥ Te ≥ TeTr; $P = 0.067$), T_C plots ($P < 0.001$), and GS_2 ($P < 0.05$). No interaction was detected for this trait at any treatment combination. When data were referred to the maximum number of exposed silks registered on day 6 in each GS × H combination (Fig. 2), it could be observed that maximum proportional silk emergence was always (i) largest and very uniform (≥83.5% of maximum) for T_C plants, and (ii) smallest for the late silking individuals of T_H plants (ranged between 51.9% for GS_2 × Tr and 78.4% for GS_1 × TeTr). A large variation was detected for this trait among T_H plants, with maximum range caused by late (51.9%) and early silking (99.1%) individuals of the Tr hybrid during GS_2 (Fig. 2f). The number of silks exposed from E_1 was reduced all along the evaluated period among late silking individuals of T_H plots, especially when heating was applied during GS_2 (Fig. 2). Heat stress reduced the proportion of florets (FPE) that reached silking (NES/FPE, Table 3), independently of the evaluated period and hybrid (0.67 for T_H and 0.79 for T_C). Similarly, it caused a significant ($P = 0.005$) increase (32.5% for T_H and 21.1% for T_C plots) in the second source of loss in kernel numbers; i.e., capacity to expose a silk from a developed floret (Table 4). This negative effect of heating was more pronounced ($P = 0.05$, Table 4) during GS_2 (15.5%) than during GS_1 (7.8%).

3.4. Final kernel number

Heat stress reduced the number of grain bearing ears per plant (prolificacy; $P < 0.001$, Table 3), and this negative effect was stronger during Exp_2 (−40% of T_C plots) than during Exp_1 (−23% of T_C plots). Interaction effects detected that this trait was (i) ≤1 in all treatment combinations (obtained as average of all surveyed plants), (ii) not affected across experiments, studied periods and hybrids for non-heated plants, and (iii) more reduced by heating at GS_2 (0.57 in Exp_1 and 0.37 in Exp_2) than at GS_1 (0.88 in Exp_1 and 0.82 in Exp_2). Additionally, it differed among hybrids in response to heating. The Te hybrid was the most sensitive (0.49 in Exp_1 and 0.45 in Exp_2), followed by the TeTr (0.76 in Exp_1 and 0.52 in Exp_2) and the Tr (0.93 in Exp_1 and 0.82 in Exp_2) hybrids (averaged of T_H plots across heating periods).

Final kernel number (KNE) was always severely ($P < 0.001$) reduced by heat stress (Table 3). Negative effects of heating were stronger during GS_2 (−68% in Exp_1 and −81.1% in Exp_2) than during GS_1 (−39.9% in Exp_1 and −63.7% in Exp_2) as compared to non-

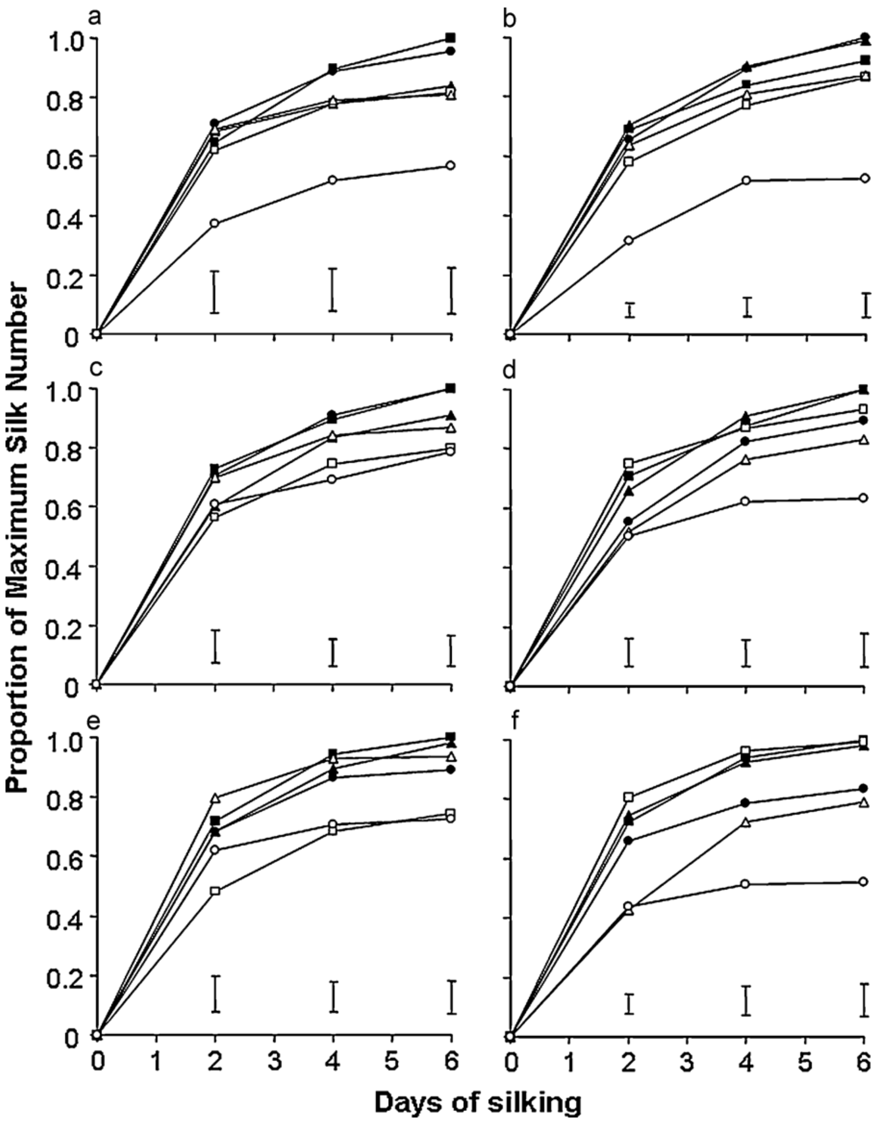

Fig. 2. Evolution of silk exposure from the apical ear of control (close symbols) and heated (open symbols) plants representative of different percentiles of the population of silking plants. Data correspond to 25% (early silking individuals, in squares), 50% (mean silking individuals, in triangles), and 75% (late silking individuals, in circles) of the population of plants. Hybrids of temperate (a and b), temperate x tropical (c and d) or tropical (e and f) background were surveyed during GS_1 (a, c, and e) and GS_2 (b, d, and f) in Experiment 2 (2009–2010). Data are expressed as a proportion of the maximum number of silks registered in each Growth Stage × Hybrid combination. Date of first silking of individual plants corresponded to day 1. Vertical bars represent the standard error of the mean.

heated plots. The interannual analysis did not detect a significant difference among hybrids in response to heating (P = 0.12 for the H × TR interaction), but within year analysis revealed a large variation (P = 0.014) during Exp_1. In this experiment, differences in KNE among hybrids were similar to those described for prolificacy. The average of T_H plots across treatment periods indicated that (i) the Te hybrid was the most affected by heating (−76.7% in Exp_1 and −77.1% in Exp_2), (ii) the TeTr hybrid had an intermediate sensitivity (−60.3% in Exp_1 and −78.1% in Exp_2), and (iii) the Tr hybrid was the less affected by this constraint (−28.1% in Exp_1 and −62% in Exp_2). Interaction effects detected that the largest drops in KNE corresponded to the Te (−93.2% in Exp_1) and the TeTr hybrids (−94.4% in Exp_2) heated during GS_2.

3.5. Kernel set

Kernel set per developed floret (KSE_1) followed the trend described for KNE and was severely reduced by heating in both experiments (P < 0.001, Table 3). In spite of no significant H × TR interaction across experiments, negative effects of heating on KSE_1 were larger for the Te (−33% across experiments) and TeTr (−30%) hybrids than for the Tr hybrid (−23%). The significant Exp × GS × TR interaction detected for this trait indicated that the negative effect of heating differed across growth stages between experiments (P = 0.014, Table 3). It was larger during GS_2 (−33%, averaged across hybrids) than during GS_1 (−16%) in Exp_1, but the opposite was verified during Exp_2 (−28% during GS_2 and −37% during GS_1). A similar trend was computed for absolute losses (Table 3). The magnitude of the decrease in this trait that could be attributed exclusively to heating was larger for GS_2 (33%) than for GS_1 (20%) in Exp_1, but did not differ between growth stages in Exp_2 (average of 39%). As for hybrids, the proportion of absolute loss was significantly (P < 0.01) smaller for the Tr germplasm (60%) than for those with temperate background (72% for the Te and 68% for the TeTr). The trend (P = 0.053) detected by the H × TR interaction highlighted that this difference was attributable to an improved performance of the Tr hybrid under heat stress (Te 89% ≅ TeTr 84% > Tr 71%), because no difference was detected in the non-heated condition (Te 54% ≅ TeTr

51% ≅ Tr 49%). Therefore, the proportion of absolute loss due to heating tended to be smaller ($P = 0.07$) for the Tr hybrid (22.7%) than for the other two hybrids (34.4% for Te and 32.5% for TeTr). Negative effects of heating were also registered for kernel set per exposed silk (KSE_2; $P < 0.001$), which ranged between 61% for T_C plots and 23% for T_H plots (Table 3).

From all computed sources of loss (Eqs. (3)–(5)), the largest magnitude (57.9%, averaged across all treatment combinations in Exp_2) corresponded to kernel abortion (Loss 3, Table 4). Failure to expose a silk from a developed floret (Loss 2) averaged 27.1% (Table 4). The magnitude of the decrease in each source of loss that could be attributed exclusively to heat effects followed the same trend: kernel abortion (38.6%) > floret failure to expose a silk (11.3%) > reduced floret differentiation in the earshoot meristem (15.3% in Exp_1 and 6.6% in Exp_2, assuming values of 0 for GS_2 in Exp_1). The evaluation of the different sources of kernel loss indicated that kernel number (i) did not respond to the proportional decrease registered in the number of florets (Fig. 3a), (ii) did respond to pollination failure ($r^2 \geq 0.69$), but independent models were necessary for adequate fit of data from each growth stage (Fig. 3b), and (iii) had a strong negative relationship with kernel abortion ($r^2 = 0.951$), well described by a single linear model (Fig. 3c).

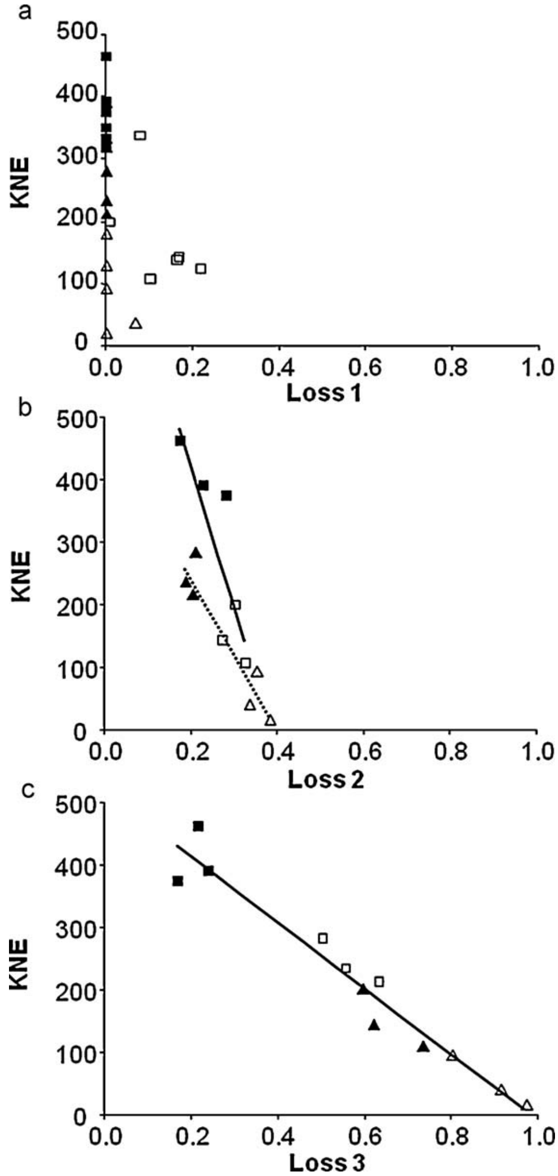

Fig. 3. Response of kernel number in the apical ear (KNE) to three sources of loss between potential and final kernel number. Loss 1 represents the decrease attributable to reductions in the number of florets per ear (a). Loss 2 corresponds to lack of pollination due to floret failure for exposing a silk (b). Loss 3 identifies kernel abortion of fertilized ovaries (c). Close and open symbols are for non-heated and heated plots, respectively. Squares and triangles identify temperature regimes imposed during the pre silking (GS_1) and the silking (GS_2) periods, respectively. Lines represent fitted linear functions. In (b) KNE = 875 − 2254 Loss 2, $r^2 = 0.69$, $P < 0.05$ (solid, for GS_1); KNE = 479 − 1198 Loss 2, $r^2 = 0.88$, $P < 0.01$ (dotted, for GS_2). In (c) KNE = 521 − 531 Loss 3, $r^2 = 0.95$, $P < 0.001$.

4. Discussion

4.1. Flowering dynamics

The observed delay in anthesis and silking dates in response to heating was opposite to the classic shortening in time to flowering in response to increased temperature; the latter has been usually reported for late sowing dates of maize crops in temperate environments (Cirilo and Andrade, 1994a; Otegui et al., 1995b). This apparent disagreement cannot be explained by means of the thermal time model based on daily mean air temperature records (Ritchie and NeSmith, 1991), which rarely includes figures above the optimum threshold in field conditions. Only models based on hourly registered temperatures (Cicchino et al., 2010a) can distinguish between below- and above-optimum figures without bias, and yield accurate cumulative stressful temperatures that do not contribute to normal crop development (as CST in Table 1). Consequences of above-optimum temperatures on flowering dynamics were a decrease in the rate of progress, a delay in flowering events and a reduction in the maximum number of plants that reached each stage (anthesis and silking). These responses took place when heating occurred during GS_1 but not when it was performed during GS_2; i.e., only when the stress matched the period of maximum tassel growth and start of active ear growth (Jacobs and Pearson, 1992b; Otegui, 1997; Uribelarrea et al., 2008) that takes place in the early phase of the critical period for kernel set (Otegui and Andrade, 2000; Westgate et al., 2004). During GS_2, lack of difference in anthesis date between temperature regimes can be attributed to the fact that tassel growth and pollen production are almost completed at this stage (Horner and Palmer, 1995; Uribelarrea et al., 2002). This is not the case for the ear, but the proportion of final ear size reached at the start of GS_2 (ca. 40% of final length in optimum growing conditions; Otegui and Bonhomme, 1998) seemed to have satisfied the minimum requirement for successful silking (Borrás et al., 2007) at all temperature regimes. Differences in flowering dynamics in response to heating between sub-periods of the critical period held across hybrids of contrasting genetic background, and are in agreement with previous research based on a single hybrid of temperate origin (Cicchino et al., 2010b).

The expected response to many abiotic stresses that take place during the late-vegetative period (i.e., GS_1) is a delay in silking date with almost no effect on anthesis date. This is attributed to the fact that organs of contrasting hierarchy within the plant (tassel ≅ uppermost internodes > ears) are undergoing active growth simultaneously at this stage (Otegui and Andrade, 2000; Westgate et al., 2004). Therefore, their relative negative response to reduced assimilate availability caused by any type of stress is opposite to their hierarchy (i.e., ears are the most affected). The consequence of this differential effect of stress is the characteristic lengthening of the interval between these events (i.e., longer ASI), extensively reported for conditions of water deficit (Hall et al., 1982; Bolaños and Edmeades, 1993) or reduced nitrogen availability (Jacobs and Pearson, 1991; D'Andrea et al., 2009). Interestingly, heat stress performed during GS_1 caused a pronounced delay in the anthesis date of all genotypes, which was even larger than previously reported for one temperate hybrid (Cicchino et al., 2010b). This delay exceeded that registered for silking, causing a decrease rather than an increase in ASI. Moreover, negative effects of heat stress on tassel growth were so drastic during GS_1 of Exp_2 that many plants never reached anthesis, a trend that hindered ASI computation. This distinctive feature of heat stress may be the direct consequence of above-optimum temperatures on anther dehiscence (Matsui and Omasa, 2002). Nevertheless, extremely reduced tassel size observed in heated plots suggested additional differential effects of high temperature on organs of contrasting position within the canopy. Those located at the top of the canopy (e.g., maize and sorghum panicles, wheat and barley spikes, sunflower capitula) are exposed to direct sunlight and experience higher temperatures than other organs (Monteith and Unsworth, 1990; Ploschuk and Hall, 1995; Ayeneh et al., 2002; Vara Prassad et al., 2006), including the ear. This condition may have resulted in a shift in sink strength for biomass allocation (i.e., reduced apical dominance), yielding a less negative effect of heating (Cicchino et al., 2010b) than of above-optimum stand density (Edmeades et al., 1993), water deficit (Echarte and Tollenaar, 2006) or nitrogen deficiency (Uhart and Andrade, 1995; D'Andrea et al., 2008) on biomass partitioning to the ear.

4.2. Floret number, silk exposure and kernel set

Floret number decreased in ears of all tested hybrids when heating was performed during GS_1. This response has been broadly documented for different types of abiotic stresses exerted during this stage (i.e., early phase of the critical period), regardless whether it was caused by above-optimum stand density (Edmeades et al., 1993; Otegui, 1997), water deficit (Hall et al., 1981; Otegui et al., 1995a), or nitrogen deficiency (Jacobs and Pearson, 1992a; Uhart and Andrade, 1995). This is the expected trend because most floret differentiation at the tip of the ear meristem takes place during this stage and does not continue after silking (Ruget and Duburcq, 1983; Fischer and Palmer, 1984; Otegui and Melón, 1997; Cárcova et al., 2003; Pagano et al., 2007). Concurrently, this trend explains the lack of effect on final floret number of heating applied during GS_2. There was, however, no correlation between floret and kernel numbers, because these traits differed markedly in the magnitude of the decrease in response to heating (much smaller for the former than for the latter) and in the stage of maximum sensitivity to stress (GS_1 for the former and GS_2 for the latter). In spite of this lack of correlation, variation in floret number allowed the detection of a differential sensitivity to high temperature among hybrids ($GS \times H \times TR$ interaction in Exp_2), which could not be attributed to non-uniform heating across experimental units (Table 1). This trend distinguished ear morphogenetic activity of the Tr hybrid as almost unaffected by above-optimum temperatures imposed in this research. By contrast, the pressence of temperate genetic background (TeTr and Te hybrids) seemed to suppress the expression of metabolic processes that helped stabilize the physiological functions of this organ under heat stress.

Heating reduced the number of exposed silks, due to mentioned negative effects on the number of florets per ear (GS_1) but also through an increased failure for exposing silks from completely developed florets (GS_1 and GS_2). Data of silk growth from experiments including above-optimum temperatures are not available for comparisons, and those obtained from ear temperature manipulation in the below-optimum range (i.e., <35 °C) indicated no effect on the silking pattern of individual plants (Cárcova and Otegui, 2001). By contrast, results from current research are supported by measurements performed on plants subjected to other abiotic stresses, which attributed the reduction in the number of exposed silks to reduced silk elongation rate (Herrero and Johnson, 1981; Jacobs and Pearson, 1991; Bassetti and Westgate, 1993c). Causes for this decrease should be sought in a decline in turgor and a restricted assimilate supply to the ear. The former is distinctive of water-limited conditions (Westgate and Boyer, 1986b; Sadras and Milroy, 1996) and does not apply to our well-watered experiments (Cicchino et al., 2010b). The latter is common to most abiotic stresses (Boyle et al., 1991; Edmeades et al., 1993; Schussler and Westgate, 1995; Echarte and Tollenaar, 2006; Pagano and Maddonni, 2007; D'Andrea et al., 2008), including heat stress (Cicchino et al., 2010b). Independently of the subjacent cause, a relevant finding of current research was the assessment of a broad variation in the silking pattern among heated plants; i.e., the reduction in the number of exposed silks varied markedly between extreme plant categories (larger in late silking individuals than in the early silking plants). Such a distinction in the silking pattern of contrasting plant categories has been seldom addressed in studies on stress physiology. The responses observed in late silking individuals of heated plots (delayed silking, reduced number of exposed silks) suggest a predominant indirect (i.e., assimilate mediated) rather than direct (e.g., due to desiccation of exposed silks) effect of heating on silk growth. First, because the position of these plants within the canopy exposed them to reduced levels of direct irradiance, with the concomitant decline in air (Monteith and Unsworth, 1990) and probably tissue (Ploschuk and Hall, 1995; Ayeneh et al., 2002; Vara Prassad et al., 2006; Rattalino Edreira et al., 2009) temperatures. Second, because the distinction among plant categories for this trait held across tested growth stages, i.e., it was independent of the presence (GS_2) or absence (GS_1) of heat stress during silking. Observed responses among plant categories are supported by evidence from hybrids with contrasting tolerance to above-optimum stand density grown at high plant populations (Pagano et al., 2007; Pagano and Maddonni, 2007). Mentioned failures in silk exposure, however, did not explain the observed variations in final kernel numbers thoroughly. No single model based on losses related to NES could fit the decline in KNE caused by the combined effects of planting date (GS_2 earlier than GS_1) and temperature regime (Fig. 3). On one hand, delayed planting produced the expected reductions in final kernel numbers (Cirilo and Andrade, 1994a; Otegui et al., 1995b), regardless of temperature regime. On the other hand, this delay was accompanied by an increased proportion of florets that did not reach silking among of T_H plots but not among T_C plots.

In spite of the clear decrease in the number of exposed silks when plants were exposed to heating, the negative trend observed in this trait was always much smaller than that registered in KNE and produced a steep decline in kernel set per exposed silk (KSE_2). A similar response has been documented for water (Hall et al., 1981; Herrero and Johnson, 1981), nitrogen (Jacobs and Pearson, 1991) and high stand density (Pagano et al., 2007) stresses, which could not be linked to negative effects of stress on pollen viability but to abortion of fertilized ovaries (Westgate and Boyer, 1986a; Otegui et al., 1995a). Heat stress always deserved a different interpretation, because negative effects on kernel set have been commonly attributed to reduced pollen viability (Herrero and Johnson, 1980; Schoper et al., 1986, 1987). In our experiments, however, the negative consequences of this constraint may be almost disregarded due to daily application of fresh pollen to all tagged plants. But most important, due to the fact that the enhanced decrease in kernel set registered among heated plants was independent of a direct effect of heating on the pollen source. It was observed when fresh pollen was applied to plants heated during pollination (GS_2) as well as to those heated before pollination (GS_1). By contrast, we detected a robust relationship between final kernel numbers and the proportion of total loss attributable to kernel abortion, which held across all tested treatments (i.e., temperature regimes, growth stages and hybrids). This relationship highlighted the occurrence of permanent negative effects of abiotic stress on maize ears and consequently on final kernel numbers. As previously demonstrated for water deficit (Otegui et al., 1995a), these effects cannot be compensated by pollen supply from a delayed pollen source (e.g., blend of hybrids in commercial maize production).

5. Conclusions

Heat stress had a negative effect on flowering dynamics and all determinants of final kernel numbers (florets per ear, exposed silks, prolificacy), but some responses did not match completely those registered for other abiotic stress (e.g., effects on anthesis date and ASI). Our most important findings were (i) the detection of permanent heat effects on the capacity of the ear for setting kernels that could not be attributed to deleterious effects on the pollen source, and (ii) important genotypic variation in the response to heating for many evaluated traits. The former identified kernel abortion as the main source of loss in kernel numbers due to heating, with a much reduced contribution from the other sources of loss (i.e., reduced floret differentiation and failure to expose a silk from a developed floret). The latter distinguished the hybrid of full tropical genetic background as better adapted to heat stress than the other hybrids (i.e., those with full or mixed temperate genetic background), but it also allowed the detection of interesting variation among traits. For instance, lack of negative heat-shock effects on floret differentiation observed in the Tr hybrid were offset by the presence of temperate background in hybrid composition. Contrary, a clear gradient was detected among hybrids in their capacity for sustaining high levels of prolificacy and final kernel numbers under heat stress (Tr > TeTr > Te).

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