

High Temperatures around Flowering in Maize: Effects on Photosynthesis and Grain Yield in Three Genotypes

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ABSTRACT

To aid breeding for heat-tolerant germplasm we analyzed the effects of high temperatures on the CO₂ exchange rate (CER), crop growth rate (CGR), kernel number (KN), and grain yield (GY) in a 30-d period bracketing flowering. Field experiments, including three maize (*Zea mays* L.) hybrids with temperate (Te), tropical (Tr) and temperate × tropical (Tx) adaptation were performed in two experiments (Exp. 1 and 2). Hybrids were subjected to high temperatures induced by shelters during a 15-d period before (H1; pre-flowering) or after silking (H2; postflowering). Crop growth rate was measured during the 30-d period bracketing silking (CGR_{CP}), H1 (CGR_{PRE}), and H2 (CGR_{POST}). Relative to nonstressed conditions, CER was reduced by 17 and 16% in H1 and H2. Moreover, CER was associated with CGR_{CP} ($r = 0.78$; $p \leq 0.001$), CGR_{PRE} ($r = 0.39$; $p \leq 0.05$), CGR_{POST} ($r = 0.51$; $p \leq 0.01$), KN (Exp. 1, $r = 0.53$; $p \leq 0.01$; Exp. 2, $r = 0.49$; $p \leq 0.01$), and GY (Exp. 1, $r = 0.59$; $p \leq 0.01$; Exp. 2, $r = 0.46$; $p \leq 0.05$). As a result of heat stress, CGR_{CP} (H1, -17%; H2, -29%), KN (H1, -7%; H2, -45%), and GY (H1, -10%; H2, -45%) were reduced relative to the control treatment. Stronger reductions for all traits in H2 relative to H1 emphasize the importance of sufficient CER during this period. The effect of high temperature on CER differed among hybrids (Tx > Te = Tr) and is promising for future germplasm screening.

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Abbreviations: ASI, anthesis–silking interval; CER, CO₂ exchange rate; CGR, crop growth rate; CGR_{CP}, crop growth rate during the critical period of ~30 day centered at silking; CGR_{POST}, crop growth rate for the 15 day after silking period; CGR_{PRE}, crop growth rate for the 15 day before silking period; DAS, days after sowing; GY, grain yield; H1, heating preflowering; H2, heating postflowering; HST, heat-stressful temperature; KN, kernel number; KW, kernel weight; Te, temperate hybrid; Tr, tropical hybrid; Tx, temperate × tropical hybrid.

HHEAT SHOCKS are likely to become more frequent as a result of global warming and are expected to have global impact (Tebaldi et al., 2006; IPCC, 2014; Eyshi Rezaei et al., 2015). Analysis of more than 20,000 historical maize yield trials in southern Africa showed that each accumulated degree-day above 30°C reduced the final yield by 1% under optimal rain-fed conditions (Lobell et al., 2011). It is therefore crucial to develop germplasm able to cope with anticipated climate change scenarios to provide sufficient food in the future. The largest negative impacts may occur in the lowland tropics, where high temperatures limit GY in maize production environments (Easterling et al., 2007).

Grain yield is the most important trait for selection in breeding programs and is closely associated with KN per unit of land area. Several studies on abiotic stresses reported an association between KN per plant and plant growth rate during a critical period of ~30 d bracketing silking when plant growth rate was modified by different plant densities (Tollenaar et al., 1992; Andrade et al., 1999;

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Vega et al., 2001), N and water deficits (Andrade et al., 2002; Roth et al., 2013), and by heat stress (Cicchino et al., 2010b; Rattalino Edreira and Otegui, 2013).

Rattalino Edreira and Otegui (2012) observed reductions in maize growth rate when exposed to brief episodes of heat stress around flowering, resulting in reduced biomass accumulation and GY. Superior performance of a tropical hybrid relative to temperate and subtropical hybrids seems to have been related to reduced rates of kernel abortion (Rattalino Edreira et al., 2011) and a stable harvest index.

A sufficiently high photosynthetic capacity in a period bracketing flowering is crucial to maintain CGR and ensure yield formation. Any reduction in photosynthesis during and after heat stress can be an indicator for thermal sensitivity (Wahid et al., 2007).

Temperatures around 38°C are expected to reduce photosynthesis in maize (Berry and Bjorkman, 1980; Crafts-Brandner and Salvucci, 2002; Naidu et al., 2003), whereas certain maize cultivars were reported to sustain photosynthesis up to 40°C (Massad et al., 2007). Improving the understanding of the physiological mechanisms that control the capacity to sustain high photosynthesis and CGR_{CP} under high temperatures is vital to improve maize yield in hot environments.

The objective of this study was to analyze the effects of high temperature treatments under field conditions during the critical period for yield determination on leaf photosynthesis, CGR, and kernel set of three maize genotypes.

MATERIALS AND METHODS

Crop Growth Conditions and Experimental Design

Field experiments were conducted at the experimental station of the Universidad Nacional del Nordeste (Corrientes, Argentina; 27°28'S; 58°49'W; 70 m above sea level). This area has a subtropical humid climate. Daily average temperatures of 25.2°C characterized the experiments during the flowering season with temperatures peaks of 43.5°C at the ear level. Experimental maize was hand planted at three seeds per hill and thinned to the desired plant population (7.5 plant m⁻²) at V3. Three sowing dates for each experiment were used to synchronize silking date between hybrids based on previous experiments. Three single-cross hybrids of contrasting genetic backgrounds (Te, Tr, and Tx) were used. Hybrids were planted on 15 (Tr), 17 (Tx), and 18 (Te) January for 2013 and on 18 (Tr), 20 (Tx), and 21 (Te) January for 2014, respectively. Rows were 0.5 m apart. Spacing between plants within a row was 0.25 m. The soil was a Hyperthermic aquic Udipsament. Soil water was kept at >50% of field capacity in the first meter of depth during the entire growing season using drip irrigation. Plots were fertilized with a total of 250 kg N ha⁻¹, 60 kg P ha⁻¹, and 199 kg K ha⁻¹ through the drip irrigation system. The fertilizer solution was applied every 3 d between 7 and 72 d after sowing (DAS). The concentration of fertigation was changed every 3 wk (Sampathkumar and Pandian, 2010), according to the following program: 7 to 28 DAS (25, 30, and 25% of total NPK, respectively), 29 to 50 DAS

(50% of total NPK), and 51 to 72 DAS (25, 20, and 25% of total NPK). The experiments were setup as a split-plot design with three replicates during 2013 (Exp. 1) and 2014 (Exp. 2). Temperature regimes were randomly assigned to main plots, while genotypes were randomly assigned to subplots within the main plots. Three thermal regimes were applied. The main plots (30 m²) were enclosed in polyethylene film shelters (7.5 m long, 4 m wide, with a max. height of 3 m), which were used to control temperature increases. The main plots were subdivided into three subplots (2.5 m long) perfectly delimited by two arches corresponding to shelter structure.

Hybrids of contrasting genetic backgrounds were planted in the subplots. Each hybrid was planted in eight linear rows 2.5 m long (subplots, 10 m²). The hybrids were DK747 VT3Pro (Te), DK390 VT3Pro (Tr), and Exp 8282 VT3Pro (Tx), all produced by Monsanto, Argentina. The VT3Pro technology offers resistance against insects, namely the corn earworm, and tolerance to glyphosate (<http://www.vttriplepro.com.ar/>). DK747 VT3Pro has been one of the most common hybrids on the Argentinian market and is characterized by its wide distribution in different maize growing areas (including the study area). Exp 8282 VT3Pro was a precommercial hybrid at the time of experimentation. DK 390 VT3Pro was widely planted in the northern region of Argentina.

Heat-Stress Treatment

High temperatures were induced during a 15-d period before silking (H1, R1 – 15 d to R1) and for a 15-d period after silking plus 2 d (H2, R1 + 2 d to R1 + 17 d). Temperatures were left at ambient levels in the control treatment. Shelters remained on the plots throughout the study and were closed during the treatment period (H1 and H2) or remained open (control). Temperatures in the H1 and H2 treatment increased between 4 and 10°C from 1000 to 1400 h during the treatment period (closed 4 h during 15 d). The roll-up shutter was kept 25 cm away from the lateral rows (i.e., rows number 1 and 8 to the west and east, respectively). The plants in rows number 1 and 8 were defoliated as needed to allow the roll up shelter to move freely. In the control plots, the roll-up shutter remained open up to 1.8 m above the soil surface. During heating in the H1 and H2 treatments, the roll-up shutter remained 0.5 m above ground to allow adequate gas exchange and avoid excessive heating.

To avoid pollen sterility and pollination constraints from heating, fresh pollen was collected every morning from plants grown under ambient conditions outside the sheltered area and used to pollinate ears with exposed silks as described by Rattalino Edreira et al. (2011). With the start of silking, manual pollinations were performed on a daily basis at 09:00 h and continued until no new silks were exposed. The arrest of silk elongation 24 h after pollination was considered evidence of a successful pollination as described previously (Bassetti and Westgate, 1993a, 1993b). For H2, shelter shutters remained open for the first 2 d after 50% of the plots reached silking to avoid heat damage to the pollen. After R1 + 2 d, heating was started in H2 plots and ears were pollinated as described above. Using this procedure, we can rule out that effects of high temperatures on GY can be biased by heat effects on pollen viability or tassel sterility.

Air temperature (Exp. 1 and Exp. 2) and relative humidity (Exp. 2) in all shelters were recorded every 5 (temperature) or

Table 1. Average, minimum, and maximum air temperatures and average relative humidity (RH) during heating. The heat effect (HE; i.e. difference between the mean on heated and control plots) and cumulative heat stressful temperatures (HST) were calculated. Fraction of time with exposure to temperatures above 34°C ($h > T_o$) also was calculated during the heating period (4 h around midday). All measurements were recorded at the ear leaf level. Heating was carried out during the presilking period (H1) and postsilking period (H2) in two experiments.

	Temperature regime	Avg.	HE	Min.	HE	Max.	HE	$h > T_o$	HST	Avg. RH
Exp. 1	C	29.0	–	22.7	–	34.0	–	6.80	62.8	–
	H1	33.1	+4.1	25.4	+2.7	39.7	+5.7	27.20	191.3	–
	H2	36.1	+7.1	24.2	+1.5	42.4	+8.4	32.40	369.0	–
Exp. 2	C	31.2	–	21.9	–	37.6	–	0.08	5.3	54.1
	H1	35.5	+4.3	24.6	+2.7	42.8	+5.2	17.00	101.8	45.8
	H2	35.9	+4.7	27.5	+5.6	43.5	+5.9	35.43	268.7	54.5

15 (humidity; Table 1) minutes throughout the treatment period using sensors connected to data-loggers (Temp and Hum-Logger, Cavadevices). These sensors were positioned in the center of each main plot at the level of the uppermost ear. Data registered in each sheltered area were used to compute average temperatures during heating (i.e., 4 h per day during 15 d). Additional temperature sensors were positioned at 1, 1.5, 2, and 2.5 m above ground to monitor air temperature throughout the canopy (only in Exp. 2). Leaf temperature was also surveyed on specific dates during the treatment period (Exp. 2) by an infrared thermometer (HI 99556, Hanna Instruments). Measurements were performed at leaf positions EL – 3, EL, and EL + 3, where EL denotes the leaf subtending the uppermost ear, and EL – 3 and EL + 3 denote three leaves below and above EL, respectively.

The heating effect was calculated as the difference in mean temperatures between control and heated plots. To identify the degree of heating, a heat-stressful temperature (HST, °C h⁻¹) was defined as in Eq. [1]:

$$\text{HST} = \sum_1^N (T_i > T_o) / (h > T_o) \quad [1]$$

Where $T_i > T_o$ represents the cumulative temperature at ear level above T_o , T_o represents the optimum temperature (34°C) proposed by Cicchino et al. (2010a), and $h > T_o$ is the number of hours with temperatures above T_o .

The CO₂ concentration was analyzed during the heating period with a portable, open-flow gas-exchange system LI-6400 (LI-COR) from a buffer box placed at 1, 1.5, 2, and 2.5 m height above ground.

Net CO₂ Exchange Rate Measurements

Leaf CER (μmol CO₂ m⁻² s⁻¹) was measured at 2000 μmol m⁻² s⁻¹ photosynthetic photon flux density at the ear leaf surface using a 6400-40 leaf chamber (LI-COR). Carbon exchange rate was measured for 2 to 5 min on a 2-cm² area of the leaf that did not include the midrib. The air flow rate through the chamber and sample side infrared gas analyzer was set to 500 μmol s⁻¹ to minimize the system response time to changes in CER. The CO₂ concentration of the intake air was maintained at 400 μmol mol⁻¹. Measurements were performed twice a day on two plants per subplot at ~1200 and 1300 h on cloudless days during at least 5 d in each period (i.e., pre- and postsilking). In all cases, steady state conditions were attained prior

to measuring gas exchange, which was reached approximately between 2 to 5 min before the start of measurement.

To assay the daily evolution of CER, additional measurements were performed on Days 7 and 14 during the daily heating period at 0, 120, and 240 min after the initiation of heating and every 30 min after the removal of heat stress (only Exp. 2).

Agronomic Traits and Sample Processing

Shoot biomass was measured destructively 15 d before silking and 15 d after silking during the critical period (Exp. 1) by harvesting five consecutive plants from rows two and seven, leaving borders between consecutive harvests. In Exp. 2, an additional harvest was performed at silking, allowing the calculation of CGR_{PRE} and CGR_{POST}. Border plants adequately surrounded sampling areas. The samples were separated into shoots and ears, oven-dried (with air circulating at 60°C) to a constant weight, and weighed. Crop growth rate during the period bracketing silking (CGR_{CP}) was calculated as the quotient of the difference of shoot dry matter produced 15 d after silking and 15 d before silking and the time elapsed between sampling. For the calculation of CGR_{PRE} and CGR_{POST}, the measurement taken at silking was used as an end or starting point, respectively.

Anthesis and silking dates were recorded when 50% of the plants within a subplot were shedding pollen and 50% of the plants exposed silks, respectively. The anthesis–silking interval (ASI) was calculated as the difference between days to silking and days to anthesis. Grain yield was determined at physiological maturity by hand harvesting 10 plants in the two center rows (rows four and five) of each subplot. Individual ears were shelled and the total number of kernels per ear determined by manual counting; results were expressed in KN per m⁻². Kernel weight (KW) was calculated as the quotient between GY and KN per plant at physiological maturity.

Statistical Analysis

Data were analyzed using a linear mixed model:

$$Y_{hjlmm} = \mu + \alpha_h + \lambda_j + \alpha_h \lambda_j + E_l + \alpha_h E_{ml} + \lambda_j E_{ml} + \alpha_h \lambda_j E_{ml} + b_m(E_{ml}) + b_m(E_{ml}) \lambda_j + r_n + e_{hjlmm}$$

where Y_{hjlmm} is the trait value of the h th genotype ($h = 3$) for the j th temperature treatment ($j = 3$; control, H1, H2), the l th experimental year ($l = 2$; 2013, 2014), the m th block ($m = 3$) and

the n th replication ($n = 3$); μ denotes the overall mean, α_h the main effect of the genotype, λ_j the main effect of the heat treatment, $\alpha_h\lambda_j$ the genotype \times heat treatment effect, E_{ml} the effect of the experimental year, $\alpha_h E_{ml}$ the genotype \times experimental year interaction, $\lambda_j E_{ml}$ the experimental year \times heat treatment effect, $\alpha_h\lambda_j E_{ml}$ the genotype \times heat treatment \times experimental year effect, $b_m(E_{ml})$ the block within experimental year effect, $b_m(E_{ml})\lambda_j$ the block within environment \times heat treatment effect, and the random error term e_{hijlmn} . The factors b_m , r_n , and E_{ml} and associated interactions were set as random, while all other factors were set as fixed. The independent two-sample t -test was used to test whether the mean temperatures of the heating treatments were significantly different from each other. Data were compared using ANOVA, Pearson's correlations among traits, and simple linear and nonlinear regression analysis for pairwise comparisons. All tests were performed with the InfoStat 2011 software (InfoStat, <http://www.infostat.com.ar>).

RESULTS

Shelter Conditions: Temperature, Relative Humidity, and CO₂ Concentration

Average temperatures recorded at the ear level were higher in Exp. 1 than in Exp. 2 because of higher ambient temperatures in the first experimental year (Fig. 1). In Exp. 1, temperature at ear level in heated plots increased 4.1°C during H1 and 7.1°C during H2 as compared with control plots (Table 1). In Exp. 2, temperature increase in heated plots was 4.3 and 4.7°C for the same periods. Average temperatures taken at ear level were not significantly different between H1 and H2 (Exp. 2, $T = 1.34$ and $p = 0.18$). However, the cumulative HSTs were 101.7 and 268.7°C h⁻¹ for H1 and H2, respectively (Table 1).

As a result of artificial heating, a gradual increase in canopy temperature from the ear to the top of the plant was observed in heated shelters (Fig. 2A). Differences between temperature regimes were also noticeable for leaf temperature records taken at three leaf positions (Fig. 2B). Relative humidity was not affected during the heating period by the temperature regime factor (Table 1). The CO₂ concentration at the top of the plant canopy (2.5 m above ground) was 310, 284, and 267 $\mu\text{mol mol}^{-1}$ for control, H1, and H2, respectively, indicating that ambient CO₂ concentration was not limiting photosynthesis (Table 2).

General Heat Effects

Different sowing dates among genotypes allowed similar silking dates. Heat treatments did not modify ($p > 0.05$) the phenology and ASI (Table 3). Temperature regimes significantly affected ($p < 0.01$) all the traits measured (except KW; Table 4). Leaf CER was reduced by 16.7 and 16.3% in H1 and H2, respectively (averaged across experiments and hybrids). At the same time, CGR_{CP} of heated plots was reduced in both treatments. Mentioned reductions relative to the control plots were greater in H2 (35.1 and 21.9% for Exp. 1 and Exp. 2, respectively) than in

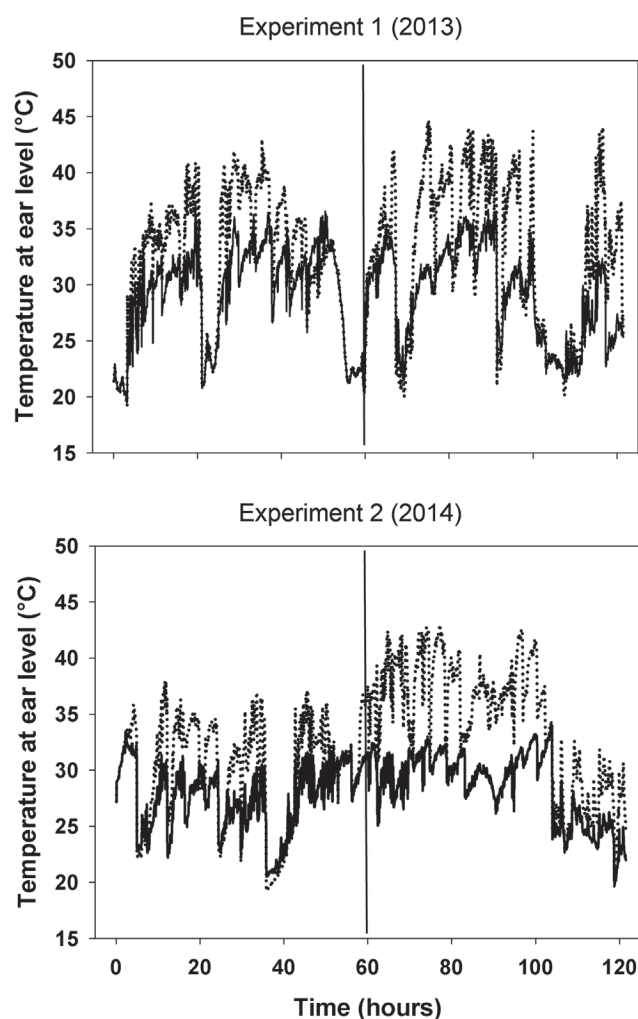


Fig. 1. Average hourly air temperature evolution at ear level of heated (dotted line) and nonheated plots (full line) during the treatment period for three maize hybrids. Vertical line represents end (H1) or start (H2) of heat treatments. Data correspond to hours when heating treatments were applied.

H1 (22.4 and 11.2%). Plants subjected to the H1 treatment seemed to have recovered after the treatment phase, as indicated by CGR_{POST} levels that were comparable with the control treatment. Although CGR_{POST} in the H1 treatment suggested a recovery, GY did not reach the same levels as the control treatment.

In accordance with reductions in CGR, the higher temperatures in H2 caused greater decreases in the KN (1546 kernels m⁻²; averaged across experiments and hybrids) than H1 (213 kernels m⁻²) relative to the nonstressed control treatment. As a result, GY was reduced by 9.8 (H1) and 44.6% (H2) relative to the control plots (averaged across experiments and hybrids; Table 4). Kernel number was associated with CGR_{CP}. Curvilinear functions of KN in dependence of CGR_{CP} were fitted for data combining hybrids, treatments, and years (Fig. 3). Kernel number dropped considerably when high temperatures were applied in the H2 treatment (−44.5% relative to control plots averaged across experiments and hybrids) compared with H1 (−6.7%).

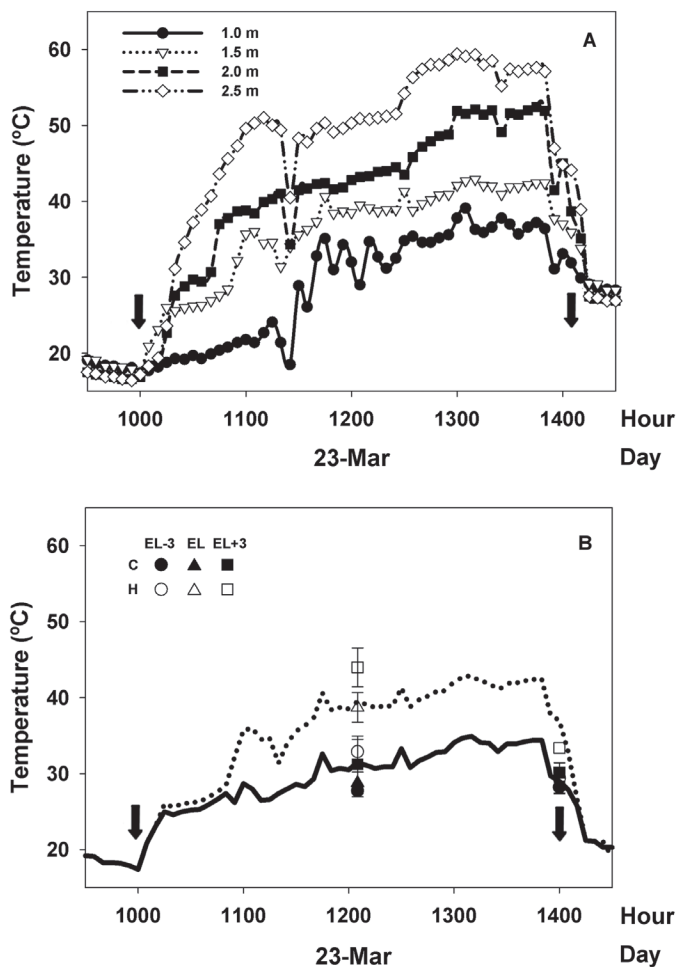


Fig. 2. Temperature evolution across canopy levels (1, 1.5, 2, and 2.5 m) within a (A) heated shelter (H2) and hourly air temperature evolution along treatment period obtained at (B) ear height. Both figures correspond to a sunny day (23 March). Arrows indicate the time when shelters were closed and reopened at the end of the heating treatment (Data for Exp. 2). Symbols in (B) correspond to leaf temperature obtained by infrared thermometry at three leaf positions: leaf subtending the uppermost ear (EL), and EL-3 and EL+3 denote three leaves below and above EL, respectively.

CO₂ Exchange Rate and its Relationship with Agronomic Traits

Leaf CER was significantly correlated with all measured traits (except KW; Table 5). Reductions in CGR_{CP} , CGR_{PRE} , and CGR_{POST} in H1 and H2 treatments (Table 4) could potentially be explained by reductions in CER, as indicated by the significant correlation (Table 5) between CGR_{CP} ($r = 0.78$ and 0.73 [$p < 0.001$] for Exp. 1 and Exp. 2, respectively), CGR_{PRE} ($r = 0.39$, $p < 0.05$, Exp. 2) and CGR_{POST} ($r = 0.51$, $p < 0.01$, Exp. 2). Negative effects of heating on the CGR can also be attributed to reductions in CER (Fig. 4). These findings were supported by the strong difference in CER between heated and nonheated plots (+5.4 and +7.1°C above max. temperatures in control treatments for H1 and H2, respectively, averaged across experiments).

Table 2. Effects of temperature regimes on average silking date and anthesis–silking interval (ASI). Data correspond to three maize hybrids (Te, temperate; Tx, temperate × tropical; Tr, tropical) under three temperature regimes: C, control; H1, heating during a 15-d period before silking (presilking period); and H2, heating from R1 + 2-d to R1 + 17-d (postsilking period). Two experimental years were analyzed (i.e. Exp. 1 and 2).

Exp.	Hybrid	Temperature regime	Sowing date	Emergence to silking	
				ASI	ASI
Exp. 1	Te	C	18 Jan. 2013	57.3 ± 0.6	1.7 ± 0.6
		H1		57.7 ± 0.6	2.3 ± 0.6
		H2		58.0 ± 1.0	1.7 ± 1.1
	Tx	C	17 Jan. 2013	58.7 ± 1.1	1.3 ± 1.5
		H1		59.7 ± 1.5	2.3 ± 1.1
		H2		59.3 ± 0.6	1.7 ± 0.6
	Tr	C	15 Jan. 2013	61.3 ± 0.6	1.0 ± 1.0
		H1		61.3 ± 0.6	1.7 ± 0.6
		H2		60.7 ± 2.1	1.3 ± 2.3
Exp. 2	Te	C	21 Jan. 2014	56.7 ± 2.1	1.3 ± 1.5
		H1		55.3 ± 2.1	1.7 ± 1.5
		H2		56.3 ± 1.5	1.7 ± 1.1
	Tx	C	20 Jan. 2014	56.7 ± 1.5	1.3 ± 1.5
		H1		58.0 ± 1.0	2.3 ± 1.5
		H2		57.0 ± 1.0	1.7 ± 1.1
	Tr	C	18 Jan. 2014	58.7 ± 1.1	1.0 ± 1.0
		H1		59.0 ± 1.0	1.3 ± 0.6
		H2		59.3 ± 0.6	1.7 ± 1.1

Table 3. Average CO₂ concentrations ([CO₂]) at different canopy levels. Data correspond to three maize hybrids (Te, temperate; Tx, temperate × tropical; Tr, tropical) under three temperature regimes: C, control; H1, heating during a 15-d period before silking (presilking period); and H2, heating from R1 + 2-d to R1 + 17-d (postsilking period). Measurements were taken at noon during heating.

Height of measurement	Temperature regime	Avg. [CO ₂]
0.5	C	362 ± 69a†
	H1	367 ± 68a
	H2	367 ± 68a
1	C	360 ± 67a
	H1	355 ± 56a
	H2	353 ± 57a
1.5	C	365 ± 61a
	H1	365 ± 67a
	H2	368 ± 68a
2	C	348 ± 49a
	H1	342 ± 46a
	H2	335 ± 31a
2.5	C	310 ± 17a
	H1	284 ± 89b
	H2	267 ± 68b

† Different letters within each column indicate significant differences ($p < 0.05$) among treatments in each canopy level.

Table 4. The effect of treatments on the CO₂ exchange rate (CER), crop growth rate (CGR), kernel number (KN), grain yield (GY), and grain weight (KW). The CER represented an average from five measurements performed during each treatment (15 d). ANOVA results are presented at the bottom of the table. The CGR_{CP} included 30 d centered in silking and CGR_{PRE} and CGR_{POST} representing 15 d before or after silking, respectively.

Exp.	Temperature regime	Hybrid	CER	CGR _{PRE}	CGR _{POST}	CGR _{CP}	KN	GY	KW
			μmol m ⁻² s ⁻¹	g m ⁻² d ⁻¹			m ⁻²	g m ⁻²	mg
Exp. 1	C	Te	37.7	–	–	33.5	2992	800.7	271.2
		Tx	35.6	–	–	34.2	3747	998.5	267.2
		Tr	35.8	–	–	40.2	3389	1055.6	303.7
	H1	Te	31.1	–	–	26.1	3003	740.8	258.9
		Tx	28.3	–	–	27.4	3119	844.3	274.4
		Tr	32.1	–	–	29.0	3684	1056.5	287.0
	H2	Te	31.4	–	–	22.2	2386	616.2	271.1
		Tx	27.2	–	–	21.7	871	315.0	289.6
		Tr	32.4	–	–	24.6	1712	505.5	298.4
Exp. 2	C	Te	33.6	32.2	33.1	32.7	3113	987.2	316.2
		Tx	34.3	31.2	41.5	36.3	3502	1162.1	329.9
		Tr	33.9	31.6	38.9	35.3	3485	1150.9	307.1
	H1	Te	30.3	27.5	36.9	32.2	2761	872.6	317.4
		Tx	25.2	24.2	33.9	29.0	3038	993.3	325.4
		Tr	27.0	26.3	35.4	30.9	3345	1024.9	335.3
	H2	Te	30.1	31.1	27.2	29.2	2430	719.0	306.0
		Tx	26.2	32.4	22.0	27.2	1412	466.3	361.0
		Tr	29.1	29.7	27.3	28.5	2140	696.1	331.8
ANOVA									
Temperature regime			<0.0001†	0.0467	0.0047	<0.0001	<0.0001	<0.0001	ns
Hybrid			<0.0001	ns‡	ns	ns	0.0283	0.0046	ns
Temperature regime × hybrid			0.0265	ns	ns	ns	<0.0001	0.0004	ns

† *p*-values of main and interaction effects.

‡ ns, not significant (*p* > 0.05).

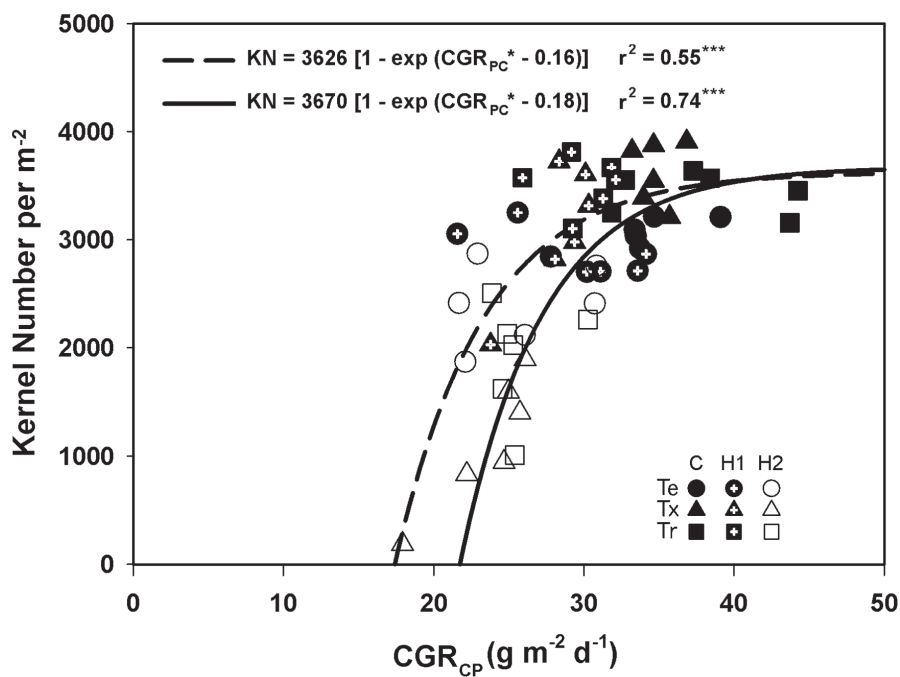


Fig. 3. Response of kernel number to variations in crop growth rate in the critical period for kernel set (CGR_{CP}). The CGR_{CP} included 30-d bracketing silking. Data correspond to three maize hybrids (circles, temperate; triangles, temperate × tropical; squares, tropical) under three temperature regimes: C, control (full symbols); H1, heating (half full symbols) during a 15-d period before silking (presilking period); H2, heating (open symbols) from R1 + 2-d to R1 + 17-d (postsilking period). Full (Exp. 1) and dotted (Exp. 2) lines represented two experimental years.

Table 5. Pearson's correlations between CO₂ exchange rate and crop growth rate (CGR), kernel number (KN), grain yield (GY), and grain weight (KW). The CGR_{CP} included 30-d bracketing silking and CGR_{PRE} and CGR_{POST} representing 15-d before or after silking, respectively. The CER measured in pre- or postsilking was correlated with CGR_{PRE} or CGR_{POST} in H1 or H2, respectively.

Agronomic traits		Exp. 1		Exp. 2	
		<i>r</i>	<i>p</i> -value	<i>r</i>	<i>p</i> -value
CO ₂ exchange rate	CGR _{CP}	0.78	<0.0001	0.73	<0.0001
	CGR _{PRE}	–	–	0.39	0.0453
	CGR _{POST}	–	–	0.51	0.0062
	KN	0.53	0.0044	0.49	0.0091
	GY	0.59	0.0013	0.46	0.0156
	KW	0.25	ns†	–0.35	ns

† ns, not significant (*p* > 0.05).

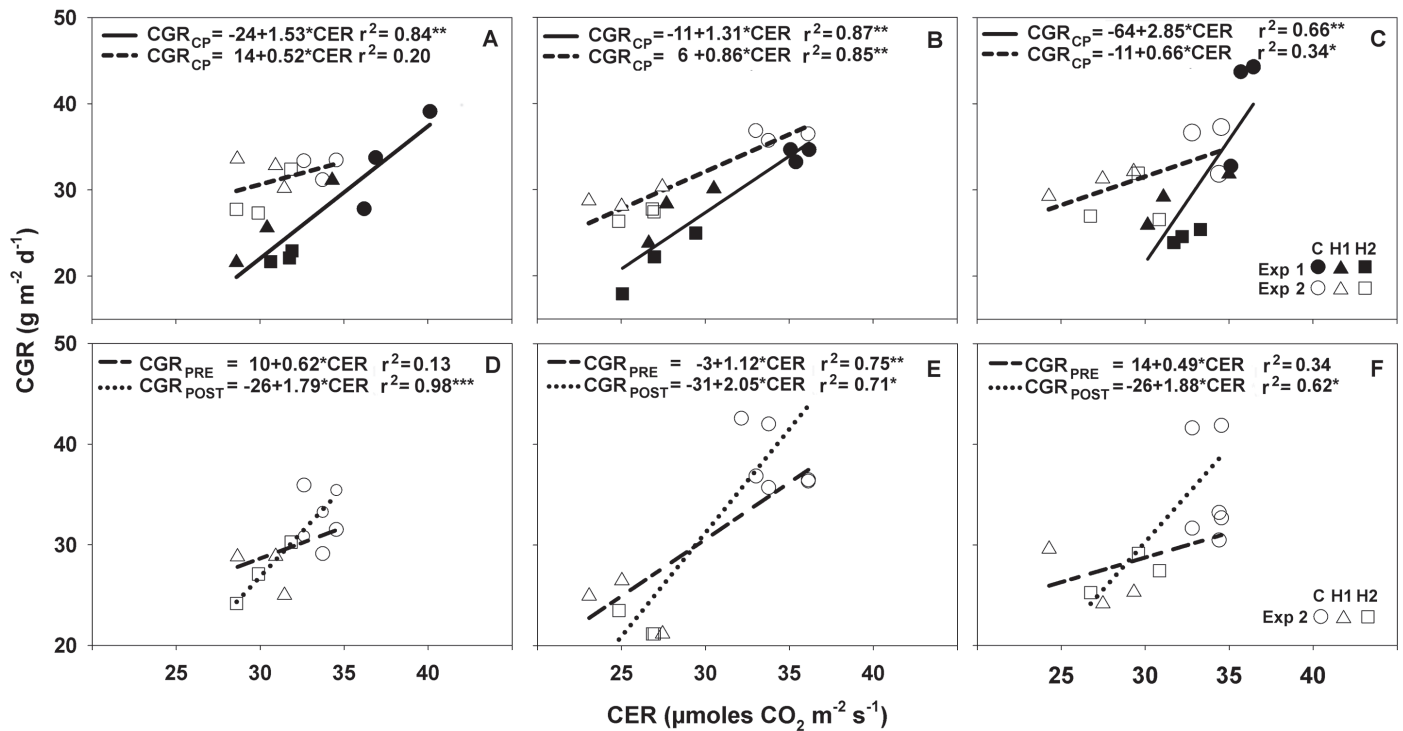


Fig. 4. Relationship between crop growth rate (CGR_{CP}, CGR_{PRE} for H1, and CGR_{POST} for H2), and CO₂ exchange rate (CER). The CGR_{CP} during a 30-d period bracketing silking and CGR_{PRE} and CGR_{POST} representing crop growth rates 15-d before or after silking, respectively. The CER displayed represents an average of five measurements performed during each treatment (15-d). Data correspond to three maize hybrids, (A and D) temperate; (B and E) temperate × tropical; and (C and F) tropical, under three temperature regimes: C, control (circles); H1, heating during (triangles) a 15-d period before anthesis (presilking period); H2, heating (squares) from R1 + 2-d to R1 + 17-d (postsilking period). Closed (Exp. 1) and open (Exp. 2) symbols represented two experimental years. In (D), (E), and (F), full and dotted lines represented CGR_{PRE} and CGR_{POST}, respectively (data only Exp. 2).

Although the response to high temperatures of KN and GY was different between H1 and H2 treatments, CER also explained the variation observed in KN through the significant correlation between KN (*r* = 0.53 and 0.49 [*p* ≤ 0.01] for Exp. 1 and Exp. 2, respectively) and GY (*r* = 0.59 [*p* ≤ 0.01] and 0.46 [*p* ≤ 0.05]), highlighting the importance of CER for kernel set and kernel formation.

Genotype × Temperature Regime Interactions

Significant temperature regime × hybrid interactions for CER (*p* = 0.03), KN (*p* ≤ 0.0001), and GY (*p* = 0.0004) indicate different responses of genotypes across treatments.

Decreases in CER relative to the control in H1 (13.6, 23.5, and 15.1% of control plots for Te, Tx, and Tr, respectively) and H2 (13.5, 23.6, and 11.7%) showed a differential genotypic response to the application of high temperatures.

Heating treatments (H1 and H2) had a similar effect on CER of fully expanded ear leaves (Fig. 5). The CER of leaves exposed to high temperatures decreased considerably relative to the control treatment depending on the genotype. Decreases in CER for Te (23.9 and 26.8% relative to the control treatment for H1 and H2, respectively) and Tr (28.4 and 26.2%) were lower than Tx (32.0 and 34.2%). This genotypic behavior depended on time of measurement during heating (i.e., 120 or 240 min) and

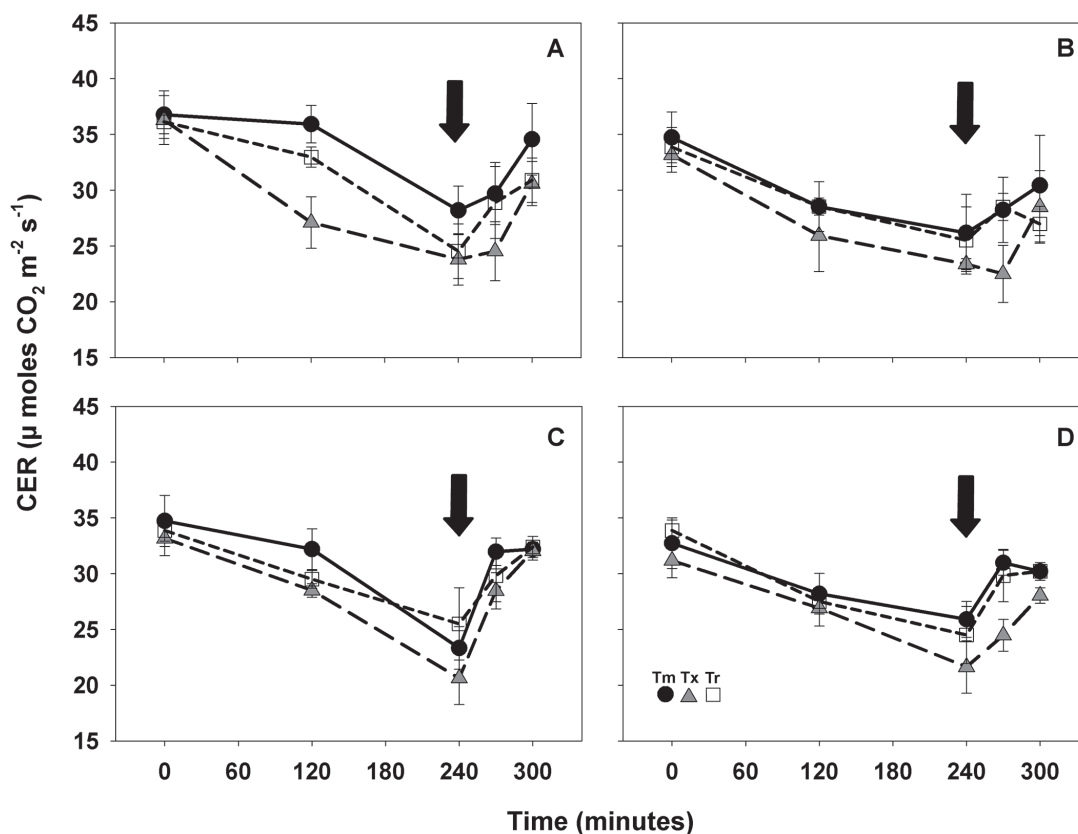


Fig. 5. Effects of heat stress and kinetic recovery on CO₂ exchange rate (CER). Data correspond to three maize hybrids (black circles, temperate; gray triangles, temperate × tropical; and white squares, tropical) under two temperature regimes: (A and B) H1, heating during a 15-d period before silking and (C and D) H2 heating from R1 + 2-d to R1 + 17-d. Arrows indicate the time when shelters were reopened at the end of the heating treatment. Measurements were performed at (A and C) 7- and (B and D) 14-d from initiating heat treatment (data only for Exp. 2).

day of heating (i.e., 7 or 14 d). After 7 d of heating (Fig. 5A, C), a significant genotypic decrease in CER was observed in the middle of the daily heating period (120 min) in both treatments (H1 and H2). However, at the end of the treatment period, there were not significant differences among hybrids during heating (14 d; Fig. 5B, D). The kinetics of recovery from heat stress (Fig. 5) indicated that photosynthesis recovered reaching 88.6% of the control treatments (averaged across hybrids) within 30 min. The CER after the daily heating period showed a significantly higher recovery in Te and Tr than in Tx (Fig. 5B, D).

The reductions in CGR_{PRE} in Tx (22.4% relative to control plots; Exp. 2) were greater in H1 than the reductions observed for Te and Tr (14.4 and 16.7%, respectively). In H2, Tx had the largest reductions in CGR_{POST} (47.2% of control plots) followed by Tr (29.9%) and Te (18.1%).

Kernel number showed a trend similar to that observed for CER. In accordance with reductions in CER, Tx showed greater reductions in KN (67.7%) than Te (20.8%) and Tr (45.2%). Grain yield was reduced by 65.2, 24.8, and 45.8%, relative to control plots under H2, and by 14.6, 7.6, and 2.6% under H1 for Tx, Te, and Tr, respectively.

DISCUSSION

Consequences of Artificial Heating

Using polyethylene shelters, the average temperature difference between heated and nonheated plots reached up to 10°C at ear level (Fig. 1). Daily heating periods were relatively short (4 h) to simulate a realistic heating regime (i.e., around midday) and to only briefly disturb the atmosphere in the heated canopy. Heated plots showed similar or lower relative humidity during the heating periods relative to the nonheated plots. Although polyethylene film decreases the amount of radiation between 10 and 12% (data not shown), polyethylene shelters were present in all treatments, and we do not expect any biasing effects by total radiation among them.

The CO₂ concentrations in plots subjected to H1 and H2 were lower than those in the control plots only at the top of the canopy. These absolute values (around 280–260 μmol mol⁻¹ at top the canopy), which are ~15% lower than those for the control treatment, could be only slightly limiting for photosynthesis in maize (Bunce, 2005). Furthermore, CO₂ concentrations were not significantly different between heated and nonheated plots at any other position in the canopy (i.e., 0.5, 1, 1.5, and 2 m; Table 2). Moreover, CER measurements at the ear level presented

intercellular airspace CO₂ concentration values above 130 μmol mol⁻¹, which are not expected to cause decreases in CER for our temperature range (Massad et al., 2007).

General Heat Effects around Silking

We propose a cumulative HST to quantify the degree of heating. We were not able to calculate optimal temperature for our experiments as a result of lack of significant effects of heat stress treatments on phenology. We used 34°C as optimum temperature (Cicchino et al., 2010a) to compute HST. This index proved to be quite useful to explain the difference between control plots across experiments, where the control plots in Exp. 1 (HST = 62.8°C h⁻¹) yielded 16% lower than control plots in Exp. 2 (HST = 5.3°C h⁻¹, averaged across hybrids).

In the current study, the ASI did not present significant differences among treatments and was even shorter than the ones reported by Neiff et al. (2015), where the average ASI reached 3 d under heat stress in 71 subtropical hybrids evaluated under field conditions.

Leaf CER values in our controls were slightly lower relative to other studies (Sadras et al., 2000; Takele and Farrant, 2013). This can be explained by differences in the measurement methodology of CER, that is, lower acclimation time. Since the method was used for all treatments obtained, we do not expect any biasing effects among treatments.

Our results showed that the daily heating around silking caused decreases in CER. During a single day of treatment, the largest decreases in CER always occurred before the opening of shelters (i.e., 240 min). During both treatment periods (i.e., 15 d), the largest decreases in CER occurred when the measurements were made at the end (i.e., 14 d) of the heat stress treatments (H1 and H2; Fig. 5).

Our results show lower average reductions on CER than on CGR_{CP}. On the one hand, average CER was computed from ear leaf measurements taken around midday, where we found significant genotypic variation but no major decreases in CER (i.e., 240 min; Fig. 5) relative to the control treatment. On the other hand, temperatures recorded at the top of the canopy were higher than those recorded at the ear level (Fig. 2A, B). Higher temperatures at the top of the canopy could potentially have more detrimental effects on CO₂ fixation (Sadras et al., 2000; Valentinuz and Tollenaar, 2004). It is therefore conceivable that CER measurements performed at midday were underestimating the CER reductions throughout the heat stress treatment.

The decreases in CGR and KN (and consequently, in GY) found in our study were associated with the effects of high temperatures on CER for both experiments, as indicated by the significant correlations between CER and CGR_{CP} ($r = 0.78$ and 0.73 [$p \leq 0.001$]; Exp. 1 and Exp. 2, respectively), KN ($r = 0.53$ and 0.49 [$p \leq 0.01$]), GY ($r = 0.59$ [$p \leq 0.01$] and 0.46 [$p \leq 0.05$]) and reductions for those traits when subjected to either heat stress treatment.

These results are in agreement with earlier studies that showed a curvilinear relationship between KN and photosynthesis at silking (Edmeades and Daynard, 1979) or by the KN and plant growth rate during the period bracketing silking (Tollenaar et al., 1992; Andrade et al., 1999; Echarte et al., 2004; Rattalino Edreira and Otegui, 2013).

We found an important recovery effect of CGR_{POST} and CER in the H1 treatment, reaching the same levels as the control treatment. These results are in accordance with Rattalino Edreira and Otegui (2012), who showed that after the removal of heat stress, plants subjected to heat stress around silking exhibited a recovery in growth and reached crop growth values similar to those registered among nonheated plants. Despite the apparent recovery of CER and CGR, this recovery did not translate into higher KN and GY. It is likely that heat stress applied before flowering resulted in the abortion of ovaries at that stage as described previously in response to stress effects (Edmeades et al., 2000) and reduced assimilate availability (Otegui and Bonhomme, 1998). Recovery of CER and CGR after removal of heat stress would not have been able to reverse this process.

Our results suggest that the effects of heat on CGR, KN, and GY are greater during the postsilking period than during the presilking period. Decreases in KN ($r = 0.81$; $p \leq 0.001$) and GY ($r = 0.85$; $p \leq 0.001$) could be explained by reductions in CGR_{POST}. Although CGR_{PRE} was affected by heat stress around silking, this trait did not present significant correlations with yield components. Negative heat effects were mainly associated with reductions in CGR pre- and postflowering as has been reported for water and N deficiencies (Muchow and Davis, 1988; Uhart and Andrade, 1995; Andrade et al., 2002) and recently for heat stress (Rattalino Edreira and Otegui, 2013).

Reductions in leaf CER during H2 resulted in a 28% reduction in CGR_{CP} during this period and reductions of at least 40% in KN and GY. The sharp decline in KN in response to decreases in CGR and the higher CGR threshold of the relationship compared with other studies (Andrade et al., 1999; Fig. 1, 2) could indicate that decreases in kernel set cannot be entirely attributed to decreases in CGR. Moreover, Rattalino Edreira and Otegui (2013) showed that losses in KN were not directly related to assimilate partitioning to the ear. These findings would indicate more direct effects of high temperatures on kernel set. According to Otegui and Bonhomme (1998), high temperatures in combination with reduced assimilate availability could have resulted in the abortion of ovaries and fertilized kernels during the 2-wk treatment period after flowering.

Potential Tolerance Mechanisms that Explain Genotypic Differences

We found larger CER decreases in Tx hybrid than in Te and Tr. The genotypic ranking is in contradiction to studies previously performed using a Te, a Tr and a Tx hybrid; Rattalino Edreira and Otegui (2012, 2013) reported inferior performance of a Te hybrid after high-temperature events compared with tropical and subtropical hybrids in a temperate region of Argentina. Causes for these discrepancies are not clear. On the one hand, a limited number of different hybrids were evaluated in both studies making it difficult to make broad inferences. On the other hand, environmental conditions (subtropical for current study vs. temperate climate for Rattalino Edreira and Otegui [2012, 2013]) used in both studies as well as the duration (4 h vs. ~12 h) and intensity of heat stress (43.5 vs. 40°C for max. temperature at the ear level during treatment period) were different in both studies potentially explaining the differences in genotypic ranking.

Although Tr and Te presented better to tolerance for heat than Tx hybrid in our experiments, under control conditions, Te yielded less than Tr and Tx. Possibly, the subtropical climate in our experiments may have lead to a potential yield penalty on Te under control conditions.

Identification of heat-tolerant germplasm has been shown to be difficult because of low heritability for GY (Weber et al., 2012) and a large genotype × environment interaction (Cairns et al., 2013). Considering the strong correlation of CER with the CGR, KN and GY, high heritability and difficulties to identify heat-tolerant germplasm (Cairns et al., 2013), CER or secondary traits associated with water homeostasis are of potential use in selection. Ongoing research using hyperspectral cameras mounted to unmanned aerial vehicles is expected to help in this field.

CONCLUSIONS

Our results indicate that CER was affected likewise by high temperatures during the presilking period relative to postsilking with varying effects on genotypes evaluated in this study. We furthermore show that reductions in CER as a result of high temperatures were directly related to variations in CGR, KN, and resulting GY, and large reduction on this trait took place when heat stress was applied in the postsilking period. Additionally, Tx was more sensitive to the effects of heat and had greater decreases in CER than Te and Tr. Correlations between CER and the other traits measured were consistent across the three hybrids with contrasting genotypic backgrounds.

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