



Heat stress in temperate and tropical maize hybrids: Kernel growth, water relations and assimilate availability for grain filling



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ABSTRACT

Several studies have indicated that maize (*Zea mays* L.) kernel weight is severely affected by heat stress, but this response was never evaluated under field conditions. Our objective was to assess the effect of brief episodes of above-optimum temperatures on the dynamics of biomass and water accumulation in kernels of maize hybrids with contrasting tolerance to heat stress. Heat effect on assimilate supply from the plant (i.e., current biomass production and water-soluble carbohydrates in stem) to developing grains was also analyzed. Field experiments included a factorial combination of (i) three hybrids (Te: temperate; Tr: tropical; TeTr: Te × Tr), (ii) two temperature regimes, control and heated during daytime hours (ca. 33–40°C at ear level), and (iii) three 15-d periods (GS1: immediately before anthesis; GS2: from silking onwards; GS3: early phase of active grain filling). Heat effects on final kernel weight were larger (i) when they occurred during the first half of effective grain filling (−23.1% for GS3) than around flowering (−4.8% for GS1, −6.3% for GS2), and (ii) for the Te hybrid (−20.4%) than for the TeTr (−8.6%) and the Tr (−6.8%) hybrids. Heating around flowering (i) enhanced the assimilate availability per kernel during the effective grain-filling period, (ii) increased carbohydrate reserves in stem at physiological maturity, (iii) and had no significant effect on the dynamics of biomass and water accumulation in kernels. The opposite trend was detected among plots heated during GS3, which mostly exhibited the interruption of grain filling. Robust associations were established between (i) carbohydrate reserves in stem at physiological maturity and assimilate availability per kernel during effective grain filling ($r^2 = 0.49$; $P < 0.001$), and (ii) the rate of water loss from kernels and the duration of effective grain filling ($r^2 = 0.71$; $P < 0.001$). These responses underlay the enhanced sensitivity to heat stress of the hybrid with full temperate genetic background.

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1. Introduction

Heat stress reduces maize (*Zea mays* L.) grain yield due to its negative effect on plant growth (Cicchino et al., 2010b) and development (Cicchino et al., 2010a). The magnitude of yield responses to above-optimum temperatures depends upon a complex function of intensity, duration, and rate of increase in temperature (Wahid et al., 2007). Further, plants sensitivity varies

along the crop cycle and differs among genotypes according to their thermotolerance level (Rattalino Edreira and Otegui, 2012). Few studies have examined the effect of heat stress on maize grain yield under field conditions. Most of them focused on the physiological determinants of grain yield (Cicchino et al., 2010b; Rattalino Edreira and Otegui, 2012) and kernel number (Rattalino Edreira et al., 2011; Rattalino Edreira and Otegui, 2013), but not on those of kernel growth. The effects of above-optimum temperatures on maize kernel growth have been studied using *in vitro* cultures of grains (Commuri and Jones, 1999; Commuri and Jones, 2001; Cheikh and Jones, 1994; Jones et al., 1984; Singletary et al., 1994), isolated plant grown in controlled-environments (Badu-Apraku et al., 1983; Wilhelm et al., 1999), or ears subject to temperature manipulations (Commuri and Jones, 2001). These studies provided information about the effect of heating on kernel ultrastructure and on metabolic processes involved in endosperm cell division and starch deposition. However, results cannot be directly extrapolated to field conditions

Abbreviations: D_{EGF}, duration of effective grain filling; Exp_n, experiment *n*; GS_n, growth stage *n*; H, hybrid; KGR_{EGF}, kernel growth rate during effective grain filling; SSR_{CP}, source-sink ratio during the critical period for kernel set; SSR_{EGF}, source-sink ratio during effective grain filling; T_C, non-heated control plot; Te, temperate hybrid; TeTr, temperate × tropical hybrid; T_H, heated plot; Tr, tropical hybrid; TR, temperature regime; WSC, water-soluble carbohydrates.

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for two main reasons. First, most heating treatments did not reproduce the daily variations in air temperature, as they usually consisted of periodic exposures to constant temperatures of variable duration. Second, the above-mentioned studies evaluated kernel growth without considering the possible effects of heating on assimilate supply from the plant to developing grains (i.e., heat effects on the source as well as on the sink).

Under non-limiting water and nutritional conditions, kernel weight is more strongly associated with the rate of kernel growth than with the duration of grain filling (Borrás and Otegui, 2001), and kernel growth rate is positively correlated with the establishment of kernel sink capacity (i.e., number of endosperm cells and amyloplasts) during the first stages of kernel growth known as *lag* phase (Capitanio et al., 1983; Reddy and Daynard, 1983). This capacity is recognized as the potential kernel weight and depends upon assimilate availability per kernel (i.e., source-sink ratio) during this phase (Lemcoff and Loomis, 1994). Plant growth rate per kernel during the critical period for kernel set (ca. 30 d around silking; Fischer and Palmer, 1984; Grant et al., 1989; Kiniry and Ritchie, 1985) has been considered a good estimator of such source-sink ratio, which is highly correlated with both kernel growth rate and potential kernel weight (Gambín et al., 2006). This conceptual framework allow us to speculate that the occurrence of a heat stress event around flowering that promotes a larger decrease in kernel set than in plant growth rate (i.e., increased source-sink ratio) may enhance the potential kernel weight. However, negative effects of heating on endosperm cell division and amyloplast biogenesis (Commuri and Jones, 1999; Denyer et al., 1994; Engelen-Eigles et al., 2001; Jones et al., 1985; Singletary et al., 1994) may limit the determination of potential kernel weight independently of assimilate availability. This suggests the existence of a trade-off between indirect and direct effects of the stress (i.e., mediated or not by assimilate availability, respectively).

Final kernel weight is also affected by growing conditions during the effective grain-filling period (Borrás and Otegui, 2001; Cirilo and Andrade, 1996). Under non-limiting conditions, the source of assimilates is usually abundant enough to cope with kernel demand, and final kernel weight do not increase much in response to enhanced assimilate availability per kernel (Borrás et al., 2004; Gambín et al., 2008). By contrast, kernel growth is highly susceptible to source limitations during this phase (Borrás et al., 2004), which reduce kernel weight by shortening of grain filling (Badu-Apraku et al., 1983; Echarte et al., 2006; NeSmith and Ritchie, 1992; Ouattar et al., 1987a; Westgate, 1994). In this situation, grain filling can be partially sustained by carbohydrate reserves in the stem (Andrade and Ferreiro, 1996; Jones and Simmons, 1983; Uhart and Andrade, 1995), which are recognized as one of the most important traits conferring tolerance to abiotic stresses (Blum, 1998; Slewinski, 2012), including heat stress (Blum et al., 1994; Tahir and Nakata, 2005; Yang et al., 2002). The contribution of this source of carbon to maize grain filling has been evaluated in a recent study on heat stress in temperate and tropical hybrids (Rattalino Edreira and Otegui, 2012). In that research, the temperate hybrid was the most sensitive to heating during effective grain filling, and its low tolerance to heating was not related to a reduced use of reserves. However, this trait was estimated as the difference between grain yield and crop biomass increase during effective grain filling, which might not reflect the actual contribution of carbohydrate reserves to biomass accumulation in the grain during the crop cycle.

The study of kernel water relations is an alternative way for assessing kernel growth dynamics. In maize, for example, some associations have been reported between (i) maximum kernel water content and kernel growth rate during effective grain filling (Borrás et al., 2003), (ii) rate of water loss from the kernel after

reaching its maximum water content and duration of grain filling (Gambín et al., 2007), and (iii) kernel moisture concentration and the onset of physiological maturity, which commonly ranges between 300 and 350 mg H₂O g fw⁻¹ (Westgate and Boyer, 1986). These relationships have been established for a wide range of genotypes (Gambín et al., 2007) and environmental conditions (Borrás et al., 2003; Sala et al., 2007b), which indicates that dry matter accumulation and water content in kernels are closely coordinated during grain filling (Schnyder and Baum, 1992). Nevertheless, source reductions during effective grain filling cause the arrest of biomass allocation to kernels and a premature decline in their water status, a condition that uncouples water from dry matter dynamics in kernels (Sala et al., 2007b). Similar responses may be expected for maize subjected to heat stress during effective grain filling due to its severe effect on plant growth (Rattalino Edreira and Otegui, 2012), but the actual impact is unknown.

The aim of the current paper was to assess the effect of brief episodes of above-optimum temperatures (e.g., less than 4 h of temperatures above 35 °C per day) during the critical period for kernel set or the first half of effective grain filling on the dynamics of biomass and water accumulation in kernels of three maize hybrids of different genetic background (temperate, tropical and temperate × tropical) grown under field conditions.

2. Materials and methods

2.1. Crop husbandry and treatments description

Field experiments were conducted during 2008–2009 (Exp₁) and 2009–2010 (Exp₂) at the experimental field of the University of Buenos Aires, Argentina (34°25'S, 58°25'W) on a silty clay loam soil (Vertic Argiudoll; USDA soil survey system). Treatments included a factorial combination of (i) three F1 hybrids (H) of contrasting genetic background (Te: temperate, Tr: tropical, and TeTr: temperate × tropical), (ii) two temperature regimes (TR) applied during daytime hours (T_C: control with no heating, T_H: heated ca. 33–40 °C at ear level), and (iii) three different growth stages (GS). Hybrids were 2M545HX (Te), 2B710HX (Tr), and 2A120HX (TeTr), all currently produced by Dow Agrosciences Argentina for different regions of this country (Rattalino Edreira et al., 2011). The relative maturities of tested hybrids were 124 for Te, 136 for Tr, and 128 for TeTr hybrids. In both experiments, a single stand density of nine plants m⁻² was used. Crops were fertilized with urea at a rate of 200 kg N ha⁻¹ at V₆ (Ritchie and Hanway, 1982). Water availability of the uppermost 1 m of the soil profile was kept near field capacity throughout the growing season by means of drip irrigation. Weeds, diseases and insects were adequately controlled. More details about crop husbandry can be found in Rattalino Edreira et al. (2011).

Treatments were distributed in a split split-plot design, with growth stages, hybrids and temperature regimes in the main plot, subplot and sub-subplot (hereafter termed plots), respectively. Three replicates were always used. Main plots were 10 m length, with six rows separated at 0.5 m between rows. Temperature regime shelters covered an area of 6 m² of the four central rows of main plots. These treatment areas were enclosed with transparent polyethylene film (100 μm thickness) mounted on 3.6-m high wood structures (Cicchino et al., 2010a). For T_C shelters, the lateral films were opened up to 1.4 m above soil surface. This was done to avoid differences in incident radiation due to the polyethylene film. For T_H shelters, the film reached the soil surface on all sides, except one side that had a 10 cm opening at the bottom. Additionally, roofs of all shelters were pierced (hole size: 0.3 cm²; holes density: 50 holes m⁻²) to avoid excessive heating in the upper part of the canopy and to allow gas exchange. Heating depended mainly on temperature rise promoted by the greenhouse effect of the polyethylene enclosure (Cicchino

et al., 2010a). Shelters for the T_H condition were supplemented with an electric fan heater (1000 W) monitored by an automated control unit (Cavadevices, Buenos Aires, Argentina) in order to increase air temperature in cloudy days.

Heating of GS_1 started when 50% of plants in control plots of each hybrid reached ca. V_{15} – V_{17} (Ritchie and Hanway, 1982; ca. 15 days before anthesis), and finished when 10% of these plants reached anthesis. Heating of GS_2 started when 10% of plants in control plots reached R_1 and finished 15 days later. Finally, the GS_3 heating period spanned from 15 days to 30 days after R_1 of control plots. All shelters were mounted and removed at the beginning and the end of each heating period, respectively. Different sowing dates were used for each $GS \times H$ combination in order to start all heating treatments at a same calendar date. This was done to achieve similar stress intensities in order to avoid the confounding effect of the environment (radiation, temperature) outside the shelters on treatments evaluation. Additionally, delayed sowing dates (from 14-November onwards for Exp_1 , and from 16-November onwards for Exp_2) were selected for starting the temperature treatments after the seasonal period of highest irradiance and temperature, which takes place between late December and the first half of January (Otegui et al., 1996). Daily mean air temperature was registered at the experimental site (Weather Monitor II, Davis Instruments, USA). Air temperature of each shelter (T_H and T_C) was recorded hourly throughout the treatment period by means of a sensor (TC1047, Microchip, US) connected to a datalogger (Temp-Logger, Cava devices, Buenos Aires, Argentina). These sensors were positioned in the center of each plot at the uppermost ear level. More details about the heating system and heat stress characteristics can be found in Rattalino Edreira et al. (2011). Additionally, we had hourly records of ambient CO_2 (GMW21, Vaisala, Finland), measured at ear level in heated and non-heated ad-hoc shelters of similar characteristics to those used for general measurements. In these shelters we also monitored air temperature (as described) and relative humidity (HIH4030, Honeywell, US) for estimating daytime VPD (Abbate et al., 2004).

Adequate pollination and fertilization of all plants was ensured in the experiments. Silks from heated plants in GS_1 and GS_2 treatments were hand pollinated between 900 and 1100 h with fresh pollen collected from non-heated plants. Pollination continued until no new silks were exposed from among the husks, and the arrest of silk elongation 24 h after pollination was taken as evidence of a successful procedure (Bassetti and Westgate, 1993a,b).

2.2. Measurements and computations

2.2.1. Source-sink ratio and final kernel weight

Forty-six plants were tagged within each sheltered area at V_{11} . Silking date (i.e., first silk visible) of the apical ear was registered for all tagged plants. Nine of them were used for estimating shoot biomass at the ontogeny stages of ca. V_{15} , R_1 and R_2 (Ritchie and Hanway, 1982) using allometric models based on the relationship between plant biomass and morphometric variables (Vega et al., 2000). For all treatment combinations, 12–15 plants of variable size (i.e., plant height, stalk diameter) were harvested at mentioned stages to estimate model parameters. Morphometric measurements included stem diameter at the base of the stalk, plant height from ground level to the collar of the last fully expanded leaf, and maximum ear diameter (only at R_1 and R_2). Models fitted to the relationship between plant biomass and morphometric variables were always significant ($P < 0.001$) and coefficients of determination averaged 0.77 across all treatment combinations. Nine plants were harvested when 50% of the grains from the mid portion of ears collected for grain filling assessment (described in Section 2.2.2) showed black layer formation (Daynard and Duncan, 1969), and final shoot biomass was registered for each plant. The

apical ear of each of these nine plants (no subapical ears were detected) was hand-shelled to determine final kernel number per plant and plant grain yield. Final kernel weight was calculated as the quotient between plant grain yield and kernel number per plant.

Individual plant biomass (i.e., estimated at V_{15} , R_1 and R_2 , and observed at physiological maturity) was used to calculate plant growth rate during the critical period for kernel set and plant biomass increment during effective grain filling. The former was computed as the slope of the linear regression fitted to estimated biomass at ca. V_{15} , R_1 and R_2 . The latter was computed as the difference between observed biomass at physiological maturity and estimated biomass at R_2 . Source-sink ratio during the critical period for kernel set (SSR_{CP}) was computed as the quotient between the plant growth rate during this period and the number of kernels per plant at physiological maturity, while source-sink ratio during effective grain filling (SSR_{EGF}) was calculated as plant shoot biomass increase per kernel during this phase. Plants with less than twenty kernels were excluded from the estimation of both SSR_{CP} and SSR_{EGF} .

The relationship between final kernel weight and SSR_{EGF} (in $mg\ kernel^{-1}$) was analyzed by means of a bilinear with plateau model (Eqs. (1) and (2)):

$$KW = a + bSSR_{EGF}, \text{ for } SSR_{EGF} \leq c \quad (1)$$

$$KW = a + bc, \text{ for } SSR_{EGF} > c \quad (2)$$

where KW is kernel dry weight (in $mg\ kernel^{-1}$), a is KW when $SSR_{EGF} = 0$ (in $mg\ kernel^{-1}$), b is the slope at the response part of the relationship (unitless), and c is the SSR_{EGF} value above which there is no change in KW (in $mg\ kernel^{-1}$).

2.2.2. Dynamics of biomass and water accumulation in the grain

Dynamics of dry matter and water accumulation in the grain during grain filling were evaluated along grain filling by means of periodic samplings of kernels from remaining tagged plants. Two plants per plot were sampled twice each week (i.e., 3–4 days between samplings) from 6–8 days after silking to 10–15 days after physiological maturity. At each sampling date, 10–15 kernels were taken from spikelet positions ten to fifteen from the bottom of the apical ear to determine kernel weights. Each ear was sampled twice at two different times (c.a., 3–4 days between sampling times). The first sample was used to determine kernel dry weight, and the second one to determine kernel dry and fresh weight and kernel volume. Grains from the first sample were collected in the field without removing the ear. This procedure consisted in opening the husks with a scalpel, extract the kernels and close the opening with adhesive paper tape after grains removal. To prevent contamination, the ear was sprayed with 70° ethanol before opening the husks. Grains from the second sample were collected in the laboratory after harvesting the ear. The apical ear with surrounding husks was harvested and enclosed in an airtight plastic bag, and immediately transported to the lab (50 m away) where it was rapidly placed in a transparent acrylic box at saturated vapor pressure (Borrás et al., 2003; Westgate and Boyer, 1986). Fresh weight and volume of kernels were measured immediately after kernels extraction in the described condition. Kernel volume was quantified by displacement of water in a graduated vial. Kernels were dried in a forced-air oven at 65 °C for at least 96 h. Fresh and dry weights were used to calculate kernel water content and kernel moisture concentration throughout grain filling.

The dynamic of kernel water content during grain filling was analyzed using a bilinear model (Eqs. (3) and (4)):

$$KWC = d + eDAS, \text{ for } DAS \leq f \quad (3)$$

$$KWC = d + ef + g(DAS - f), \text{ for } DAS > f \quad (4)$$

where KWC is the kernel water content (in mg kernel⁻¹), DAS is days after silking, d is the kernel water content at silking (in mg kernel⁻¹), e is the rate of water accumulation in the kernel before achieving maximum water content (in mg kernel⁻¹ d⁻¹), f is the time at maximum water content (in d), and g is the rate of water loss from the kernel after reaching maximum water content (in mg d⁻¹). Maximum kernel water content was computed as the water content value at f .

The rate and duration of effective grain filling were estimated by fitting a trilinear model to kernel weight data (Eqs. (5–7)):

$$KW = KGR_{lag} DAS, \text{ for } DAS \leq D_{lag} \quad (5)$$

$$KW = KGR_{lag} D_{lag} + KGR_{EGF} (DAS - D_{lag}), \text{ for } D_{GF} \geq DAS > D_{lag} \quad (6)$$

$$KW = KGR_{lag} D_{lag} + KGR_{EGF} (D_{GF} - D_{lag}), \text{ for } DAS > D_{GF} \quad (7)$$

where DAS is days after silking, KGR_{lag} is the kernel growth rate during the *lag* phase (in mg kernel⁻¹ d⁻¹), D_{lag} is the duration of the *lag* phase (in d), KGR_{EGF} is the kernel growth rate during effective grain filling (in mg kernel⁻¹ d⁻¹), and D_{GF} is the duration of grain filling (in d). The duration of effective grain filling (D_{EGF} in d) was calculated as the difference between grain-filling duration and *lag* phase duration (i.e., $D_{GF} - D_{lag}$).

2.2.3. Water-soluble carbohydrates in stem

In Exp₂, water-soluble carbohydrates in stem were measured fortnightly from ca. V₁₅ to physiological maturity. At each sampling date, 1–2 plants per plot (3–4 plants at physiological maturity) were cut at ground level and stem plus sheaths (this set will be hereafter termed stem) were separated from the rest of the plant. The stem of each plant was oven dried until constant weight, weighed and ground. Water-soluble carbohydrates were measured in extracts from 0.1 g of dry tissue according to the methodology of Yemm and Willis (1954). The amount of water-soluble carbohydrates (WSC) accumulated in the stem was calculated as the product of stem dry weight and the concentration of water-soluble carbohydrates in stem.

The relationship between WSC in stem and SSR_{EGF} was evaluated by using a bilinear with plateau model (Eqs. (8) and (9)):

$$WSC = h + iSSR_{EGF}, \text{ for } SSR_{EGF} \leq j \quad (8)$$

$$WSC = h + ij, \text{ for } SSR_{EGF} > j \quad (9)$$

where WSC is the amount of water-soluble carbohydrates accumulated in stem (in g plant⁻¹), h is the WSC in stem when $SSR_{EGF} = 0$ (in g plant⁻¹), i is the slope at the response part of the relationship (in g kernel plant⁻¹ mg⁻¹), and j is the SSR_{EGF} value above which there is no change in WSC in stem (in mg kernel⁻¹).

2.3. Statistical analysis

The effect of treatments and their interactions was analyzed for all described traits by analysis of variance performed across experimental years (Grupo InfoStat, 2010). The model for the split split-plot design is described in Eq. (10):

$$Y_{ijkl} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \gamma_k + \alpha\gamma_{ik} + \beta\gamma_{jk} + \alpha\beta\gamma_{ijk} + \tau_1 + \alpha\tau_{i1} + \beta\tau_{j1} + \gamma\tau_{k1} + \alpha\beta\tau_{ij1} + \beta\gamma\tau_{jk1} + \alpha\beta\gamma\tau_{ijk1} + \varepsilon_{ijkl} \quad (10)$$

where each measured trait (Y_{ijkl}) can be described as the overall mean (μ) plus an experiment effect (α_i), a main plot effect (β_j), a sub-plot effect (γ_k), a sub-sub-plot effect (τ_1), interactions among them and an error term (ε_{ijkl}). A t -test was used to detect significant differences ($P < 0.05$) among means. Linear regression

was used to test the relationship between variables, and models (Eqs. (1–9)) were fitted using Table Curve (Jandel-Scientific, 1991) software.

3. Results

3.1. Growing conditions during grain filling and the heating period

Detailed information on meteorological conditions during experiments can be found in Rattalino Edreira et al. (2011) and Rattalino Edreira and Otegui (2012). Briefly, different experimental years and sowing dates caused little variations in the thermal regime during grain filling. Mean air temperature was slightly higher in Exp₁ (23.9 °C; averaged of control plots across GS × H combinations) than in Exp₂ (23.4 °C) and the most delayed sowing (i.e., GS₁) exposed the crop to lower temperatures (22.5 °C, averaged of control plots across Exp × H combinations) than the intermediate (23.9 °C) and early (24.5 °C) sowings.

Heating increased air temperature at ear level during the treatment period, especially around midday (Rattalino Edreira and Otegui, 2012). Differences in this variable between heated and control plots were 4.61 °C from 1100 to 1600 h and 0.33 °C for the rest of the day (averaged across GS × H combinations and experiments). Therefore, heating had an important effect only on maximum air temperature at ear height (35.2 ± 3.5 °C for heated plots and 30.2 ± 3.3 °C for control plots; average of daily absolute maximum temperature during the heating period across all treatment combinations), but not on mean air temperature at ear height (24.7 ± 0.9 °C for heated plots and 23.4 ± 1.3 °C for control plots; average of daily mean temperature during the heating period across all treatment combinations). Within each experiment, the intensity of heat stress was similar for each GS × H combination, but it was larger for Exp₁ (36.1 °C) than for Exp₂ (34.8 °C).

CO₂ values surveyed in the ad-hoc plots were never lower than 225–250 ppm for T_H and 300–325 ppm for T_C, and daytime VPD values ranged between 0.019 and 1.386 kPa for T_H (mean = 0.93 ± 0.41) and 1.115 and 1.928 kPa for T_C (mean = 1.44 ± 0.255).

3.2. Final kernel weight and grain filling

Final kernel weight differed ($P < 0.001$) between experimental years and among genotypes, but not among sowing dates (i.e., GS treatments) when the analyses considered only the non-heated plots. This variable was higher in Exp₁ (298 mg kernel⁻¹; averaged across treatment stages and hybrids) than in Exp₂ (260 mg kernel⁻¹), and was slightly higher for the TeTr hybrid (299 mg kernel⁻¹, averaged across experiments and treatment stages) than for the Te (275 mg kernel⁻¹) and Tr (263 mg kernel⁻¹) hybrids. Kernel weight variation of non-heated plots was partially explained by the variation in kernel growth rate during effective grain filling ($KW = 97 + 25.4 KGR_{EGF}$; $r^2 = 0.34$; $P = 0.01$), but not by the variation in effective grain-filling duration ($KW = 116 + 4.1 D_{EGF}$; $r^2 = 0.07$; $P = 0.09$).

Heat stress reduced ($P < 0.001$) final kernel weight, but the magnitude of this effect varied among hybrids and treatment stages (Table 1). In general, the Te hybrid was more affected by heating (−20.4%, averaged across experiments and treatment stages) than the TeTr (−8.6%) and Tr (−6.8%) hybrids. Genotypes were more sensitive to heating during effective grain filling (−23.1% for GS₃ respect to non-heated plots; averaged across experiments and hybrids) than during the period around flowering (−4.8% for GS₁ and −6.3% for GS₂). The significant Exp × GS × H × TR interaction ($P = 0.007$; Table 1) detected for this variable indicated that the largest reduction in final kernel weight corresponded to the Te hybrid heated during GS₃ in Exp₁ (−52%). Observed variation in final kernel weight of heated plots pooled across treatment stages was

Table 1

Treatment effect on kernel number per plant, final kernel weight, rate (KGR_{EGF}) and duration (D_{EGF}) of effective grain filling, maximum kernel water content, rate of water loss from kernels after reaching maximum water content, kernel moisture content at physiological maturity, maximum kernel volume, source-sink ratio during the critical period for kernel set (SSR_{CP}) and source-sink ratio during effective grain filling (SSR_{EGF}). Summary ANOVA table at the floor of Table 1 shows significance levels for treatment effects and their interactions.

Exp	GS	H	TR	Kernel number (n°. plant ⁻¹)	Kernel weight (mg kernel ⁻¹)	KGR_{EGF} (mg d ⁻¹)	D_{EGF} (d)	Maximum water content (mg kernel ⁻¹)	Rate of water loss (mg d ⁻¹)	Moisture content at R_0 (mg g ⁻¹)	Maximum volume (μl kernel ⁻¹)	SSR_{CP} (mg d ⁻¹ kernel ⁻¹)	SSR_{EGF} (mg kernel ⁻¹)
Exp ₁	GS ₁	Te	T _C	351	297	6.9	42	196	-1.8	372	383	15	101
			T _H	140	277	7.1	38	200	-1.8	417	351	21	449
		TeTr	T _C	320	340	8.1	42	204	-1.3	366	432	14	261
			T _H	125	308	8.7	35	216	-2.7	396	421	26	590
		Tr	T _C	334	298	6.4	44	196	-1.0	401	375	14	163
			T _H	339	291	6.2	46	187	-1.1	378	373	8	262
	GS ₂	Te	T _C	337	280	6.3	44	194	-1.3	386	372	16	56
			T _H	23	242	- ^a	-	-	-	-	-	27	309
		TeTr	T _C	322	309	8.3	36	193	-2.4	373	402	17	58
			T _H	130	285	8.2	35	195	-2.4	362	405	26	362
		Tr	T _C	392	248	6.2	41	168	-2.3	386	349	11	110
			T _H	183	242	6.2	42	168	-1.9	405	401	21	288
	GS ₃	Te	T _C	439	288	7.2	38	179	-1.7	382	385	10	196
			T _H	369	139	7.2	17	172	-5.3	547	264	13	-42
		TeTr	T _C	388	328	8.4	40	207	-2.5	333	415	13	201
			T _H	350	310	7.9	40	188	-1.9	332	382	15	181
		Tr	T _C	383	298	7.3	40	161	-1.8	355	360	11	185
			T _H	239	236	6.0	35	157	-3.6	403	281	16	72
Exp ₂	GS ₁	Te	T _C	392	282	6.9	42	194	-1.7	361	399	12	216
			T _H	108	235	8.6	32	192	-3.0	421	370	27	594
		TeTr	T _C	375	260	8.0	36	197	-2.2	384	393	9	253
			T _H	144	259	8.5	33	202	-2.3	416	413	19	438
		Tr	T _C	464	252	6.5	42	175	-2.0	356	383	8	273
			T _H	200	267	6.5	43	196	-2.1	346	410	9	442
	GS ₂	Te	T _C	213	254	7.0	40	199	-2.0	387	422	17	255
			T _H	39	268	7.6	33	212	-2.3	365	354	20	449
		TeTr	T _C	234	279	7.6	39	188	-1.9	369	403	12	322
			T _H	13	238	-	-	-	-	-	-	31	722
		Tr	T _C	283	266	6.5	38	161	-2.6	385	391	8	305
			T _H	93	237	7.2	35	182	-3.0	409	350	14	553
	GS ₃	Te	T _C	406	251	7.2	36	185	-2.2	381	358	9	185
			T _H	391	174	7.6	23	184	-3.3	457	312	10	-1
		TeTr	T _C	318	276	7.5	39	175	-3.3	317	361	13	188
			T _H	317	232	7.4	34	178	-2.8	346	328	12	108
		Tr	T _C	450	216	6.6	35	173	-2.5	396	342	6	159
			T _H	389	187	5.8	36	155	-2.3	384	298	9	103
Experiment (Exp)	ns			0.001 ^b	ns	ns	ns	0.003	ns	ns	ns	ns	0.004
Growth stage (GS)	<0.001			<0.001	ns	0.008	<0.001	<0.001	ns	<0.001	<0.001	<0.001	<0.001
Hybrid (H)	<0.001			<0.001	<0.001	<0.001	<0.001	0.013	<0.001	0.003	0.005	0.039	0.039
Temperature regime (TR)	<0.001			<0.001	ns	<0.001	ns	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Exp × GS	ns			0.010	ns	ns	ns	0.010	ns	ns	ns	ns	<0.001
Exp × H	ns			<0.001	0.046	ns	0.002	0.003	0.034	ns	ns	ns	ns
Exp × TR	ns			ns	ns	ns	ns	0.010	ns	ns	ns	ns	ns
GS × H	0.008			<0.001	ns	<0.001	0.015	<0.001	<0.001	ns	ns	ns	ns
GS × TR	<0.001			<0.001	ns	ns	0.009	0.020	0.008	0.001	<0.001	<0.001	<0.001
H × TR	ns			0.001	ns	<0.001	ns	<0.001	<0.001	0.010	ns	ns	ns
Exp × GS × H	0.035			0.020	ns	ns	ns	<0.001	0.026	ns	ns	ns	ns
Exp × GS × TR	0.002			ns	ns	ns	ns	0.004	ns	0.019	ns	ns	ns
GS × H × TR	ns			0.001	ns	ns	ns	<0.001	0.005	ns	ns	ns	0.037
Exp × GS × H × TR	ns			0.007	ns	ns	0.017	<0.001	ns	ns	ns	ns	ns

ns: not significant ($P > 0.05$).

^a Missing values correspond to treatment combinations for which heating caused a severe reduction in prolificacy (grained ears per plant) and kernel numbers that did not allow for a correct survey of several physiological traits related to grain filling.

^b P values of main and interaction effects for which at least one variable was detected as significant.

attributable to changes in effective grain-filling duration ($KW = 69 + 5.1 D_{EGF}$; $r^2 = 0.56$; $P < 0.001$), and not to changes in kernel growth rate during effective grain filling ($KW = 143 + 14.2 KGR_{EGF}$; $r^2 = 0.09$; $P = 0.29$). This response was strongly influenced by the severe shortening of grain-filling duration due to heat stress

during GS₃ (Table 1), as neither the duration ($KW = 224 + 1.14 D_{EGF}$; $r^2 = 0.05$; $P = 0.53$) nor the kernel growth rate ($KW = 116 + 4.1 KGR_{EGF}$; $r^2 = 0.13$; $P = 0.14$) during the effective grain-filling period accounted for the variation in final kernel weight when the analysis included only plots heated around flowering (GS₁ and GS₂).

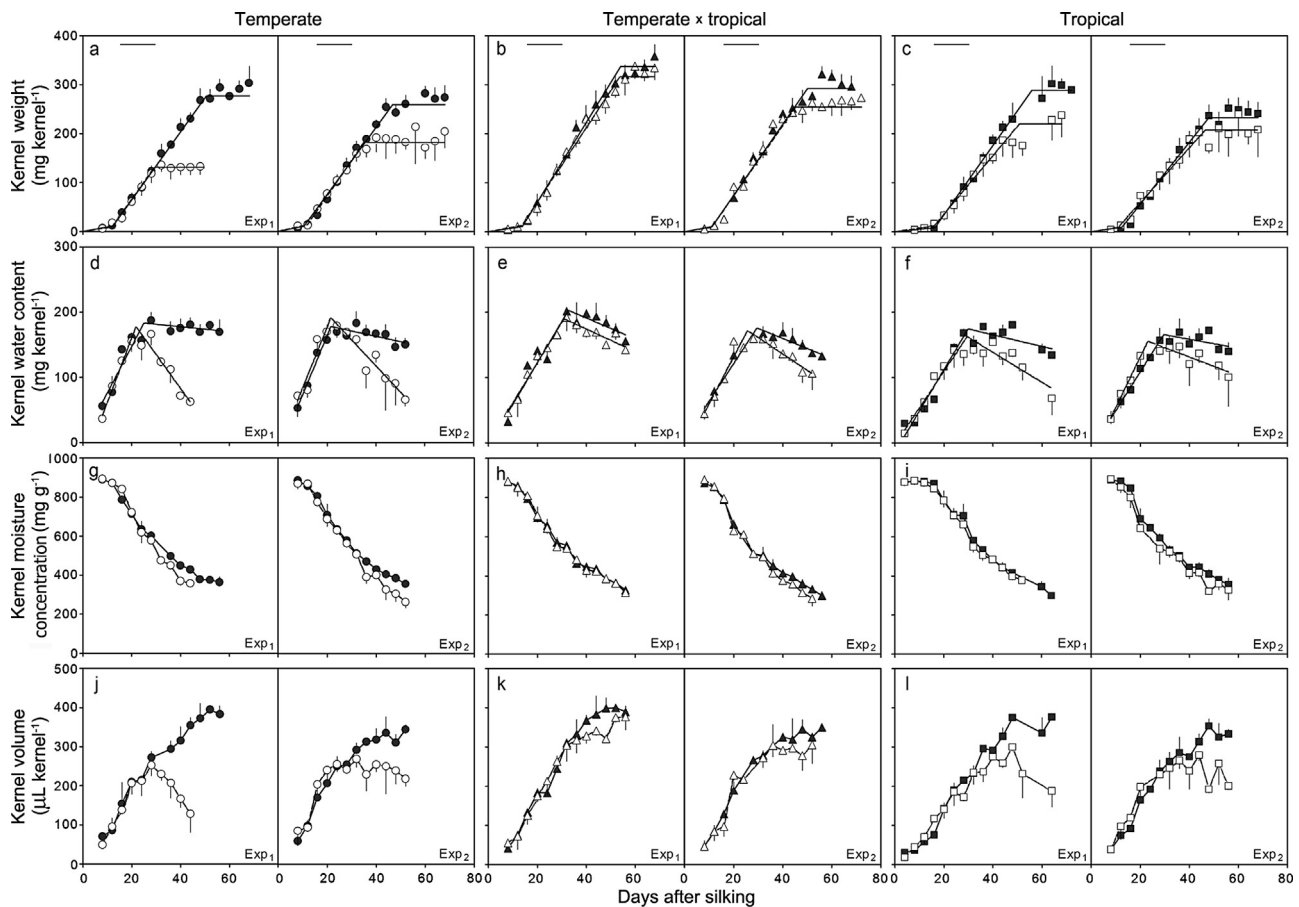


Fig. 1. Evolution of kernel weight (a–c), kernel water content (d–f), kernel moisture concentration (g–i), and kernel volume (j–l) of temperate (circles), temperate × tropical (triangles), and tropical (squares) hybrids exposed to heated (open symbols) and non-heated (closed symbols) conditions during the first half of effective grain filling (GS₃) in two experimental years (Exp_n). Lines in (a–c) and (d–f) represent models fitted to kernel dry weight and kernel water content data. Horizontal bars in (a–c) indicate the duration of the heating period. Most of the parameters values for these fitted models are detailed in Table 1. Vertical bars represent SE values.

Shortening of grain filling was more marked for the Te hybrid than for the other hybrids (–12.2 d for Te, –3.2 d for TeTr, and –0.5 d for Tr; averaged across experiments and treatment stages), and the greatest shortening (–21 d) was observed for the Te hybrid heated during GS₃ in Exp₁ (Fig. 1a–c).

3.3. Kernel water relations and kernel volume

Heat stress around flowering had little effect on kernel growth (as reported in Section 3.2), kernel water relations and kernel volume, but these traits were strongly affected by heating during the first half of effective grain filling (Table 1). Therefore, the dynamics of dry matter accumulation (Fig. 1a–c), water content (Fig. 1d–f), moisture concentration (Fig. 1g–i), and volume of kernels (Fig. 1j–l) were plotted only for GS₃. Kernel water content along grain filling was similar among hybrids and sowing dates in non-heated plots. Heat stress caused considerable changes in such pattern by increasing the rate of water loss from kernel (in absolute terms) after reaching the maximum water content, but not by changing the maximum kernel water content (Table 1). The magnitude of this increase was larger for heating during the first half of effective grain filling (53% for GS₃ respect to non-heated plots; averaged across experiments and hybrids) than for heating around flowering (33% for GS₁ and 3% for GS₂), being the Te hybrid the most sensitive to heating during GS₃ (Fig. 1d–f). The duration of effective grain filling was linearly and positively related ($D_{EGF} = 51 + 5.8 g$; $r^2 = 0.71$; $P < 0.001$) to the rate of water loss from kernel (parameter g in Eq. (4)) after reaching the maximum water content.

Kernel moisture concentration decreased gradually throughout grain filling in non-heated plots, following a similar pattern among stages and hybrids. On average, hybrids reached physiological maturity when kernel moisture concentration was 378 mg H₂O g fw⁻¹ for Te, 357 mg H₂O g fw⁻¹ for TeTr, and 380 mg H₂O g fw⁻¹ for Tr (averaged of non-heated plots across experiments and treatment stages). Heat stress around flowering (GS₁ and GS₂) had no effect on the dynamics of kernel moisture concentration (data not shown), but this pattern was affected by heating during the first half of grain filling (GS₃, Fig. 1g–i). The negative effect of the stress on grain-filling duration caused an increased kernel moisture concentration at physiological maturity and enhanced kernel desiccation rate (mg H₂O g fw⁻¹) after the end of grain filling (Fig. 1d–f). This effect was more evident when the stress caused a severe shortening of grain-filling duration. Therefore, the highest moisture concentration value at physiological maturity (547 mg H₂O g fw⁻¹) and the largest kernel desiccation rate were observed for the Te hybrid heated during GS₃ in Exp₁ (Fig. 1g).

Kernel volume in non-heated plots increased throughout grain filling and reached its maximum value close to physiological maturity (Fig. 1j–l). Maximum kernel volume varied between 342 and 432 μL kernel⁻¹ across non-heated treatment combinations (Table 1). Heating around flowering had no effect on this trait (–2% for GS₁ and 0% for GS₂ respect to non-heated plots; averaged across experiments and hybrids), but maximum kernel volume was strongly reduced by heating during the first half of effective grain filling (–16% for GS₃; Fig. 1j–l). The Te hybrid had a larger

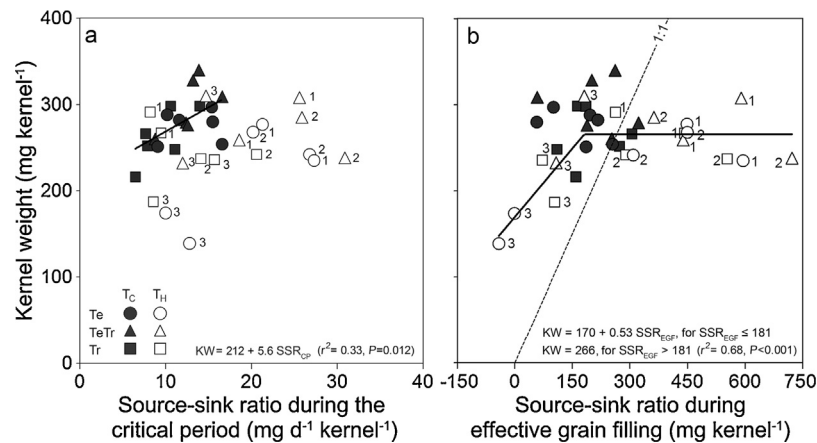


Fig. 2. Relationship between kernel weight (KW) and source-sink ratio during (a) the critical period for kernel set (SSR_{CP}), and (b) the effective grain-filling period (SSR_{EGF}) of three maize hybrids of contrasting origin (Te: temperate; Tr: tropical; TeTr: Te × Tr) exposed to two temperature regimes (T_C: control; T_H: heated during daytime hours) during three growth stages (GS₁: 1; GS₂: 2; GS₃: 3; stages only identified for heated plots) in two experimental years. Each GS covered a 15-d period (GS₁: immediately before anthesis; GS₂: from the start of silking onwards; GS₃: from the end of GS₂ onwards). Equation and solid line correspond to the model fitted to data from non-heated plots (a) or heated plots (b). Dashed line in (b) represent the 1:1 ratio between variables. Each symbol represents the mean of three replicates within each experimental year.

reduction in maximum kernel volume (−22% respect to non-heated plots; averaged across experiments) due to heating during GS₃ than the TeTr (−9%) and the Tr (−17%) hybrids. In addition, shriveled grains were observed at physiological maturity for the Te hybrid in Exp₁ (visual assessment) as a result of severe reduction in kernel volume after reaching its maximum volume.

3.4. Source-sink relationships and kernel weight

Heat stress affected plant growth and kernel set depending on the time of the stress, which produced a wide variation in source-sink ratios. Detailed information about the negative effect of heating on plant growth and kernel set can be found in [Rattalino Edreira and Otegui \(2012; 2013\)](#). Briefly, heat stress around flowering (GS₁ and GS₂) had a negative effect on plant growth rate during the critical period for kernel set, while heating during GS₃ reduced plant growth during effective grain filling. The Te hybrid tended to have the largest reductions in both traits (Te ≥ Tr ≥ TeTr) as a result of above-optimum temperatures. Final kernel number ([Table 1](#)) was severely reduced by heating around flowering, but this trait was not affected by heating during the first half of grain filling (except for the Tr hybrid in Exp₁). Heating around flowering caused a larger reduction in kernel number per plant for the Te hybrid (−66% for GS₁ and −87% for GS₂ respect to T_C plots; averaged across experiments) than for the TeTr (−61% for GS₁ and −77% for GS₂) and Tr (−28% for GS₁ and −60% for GS₂) hybrids.

Plant growth rate per kernel during the critical period for kernel set (SSR_{CP}) in non-heated plots was similar among hybrids and treatment stages. Heating around flowering caused significant increases in this variable due to an effect on kernel number ([Table 1](#)). However, SSR_{CP} explained a small part of the variation registered in kernel weight of non-heated plots ($r^2 = 0.33$; $P = 0.012$), whereas no trend was registered between these traits among those exposed to heating. Nevertheless, most data from the latter fell below the relationship fitted to control plots ([Fig. 2a](#)). Plant growth per kernel during effective grain filling (SSR_{EGF}) ranged between 57 and 322 mg kernel⁻¹ across all non-heated treatment combinations ([Table 1](#)). Heat stress affected SSR_{EGF} , either positively or negatively, depending on the time of occurrence of the stress, which increased the range of explored SSR_{EGF} values (between −42 and 722 mg kernel⁻¹). While heating around flowering had a positive effect on SSR_{EGF} (140% for GS₁ and 235% for GS₂; averaged across experiments and hybrids), this trait

was reduced by heating during the first half of effective grain filling (−62% for GS₃). On average, the Te hybrid had the largest increase in SSR_{EGF} when heating was performed around flowering (261% for Te, 210% for TeTr and 91% for Tr; averaged of GS₁ and GS₂ across experiments) and the largest decrease in this trait when heating occurred during the first half of grain filling (−111% for Te, −26% for TeTr and −61% for Tr; averaged across experiments).

Final kernel weight had a bilinear with plateau response ($r^2 = 0.41$; $P < 0.001$) to variations in SSR_{EGF} (Eqs. (1) and (2)). However, the data set did not have a random distribution of residuals, which were positive (189 ± 30) for control plots and negative for the heated ones (-189 ± 30). When temperature regimes were analyzed separately, a significant fit ($r^2 = 0.68$; $P < 0.001$) could be established only for the latter ([Fig. 2b](#)), with a breakpoint at 170 mg kernel⁻¹ for reaching maximum kernel weight in this condition. Below this threshold value, kernel weight declined at a rate of -0.53 mg kernel⁻¹ per unit of decline in SSR_{EGF} .

3.5. Water-soluble carbohydrates in stem

The amount of water-soluble carbohydrates (WSC) accumulated in stems was always low at the onset of the critical period for kernel set, ranging between 1 and 6.4 g plant⁻¹ across all treatments combinations. From this time onwards, the pattern of WSC accumulation and remobilization in non-heated plots differed among sowing dates and, to a lesser extent, among hybrids within each sowing date ([Fig. 3](#)). Late (GS₁) and intermediate (GS₂) sowings showed sustained increases in WSC in stems during grain filling, while early sowing (GS₃) experienced minimum changes in this trait from ca. 10–20 days after silking onwards.

Heat stress tended to reduce WSC in stem during the heating period ([Fig. 3](#)). However, this effect was enhanced when the stress was performed late in the crop cycle (GS₃ > GS₂ > GS₁), and there were many cases for which significant differences were not detected between heated and non-heated plots during treatment period ([Fig. 3a–d](#)). After heat stress removal, plants subjected to heating around flowering tended to increase WSC in stem respect to their non-heated counterparts and, in most cases, WSC at physiological maturity were higher for heated than for non-heated plants ([Fig. 3a–f](#)). By contrast, heat stress during the first half of effective grain filling caused sustained losses of WSC in stem, reaching minimum values close to physiological maturity ([Fig. 3g–i](#)). This trend was particularly pronounced for the Te

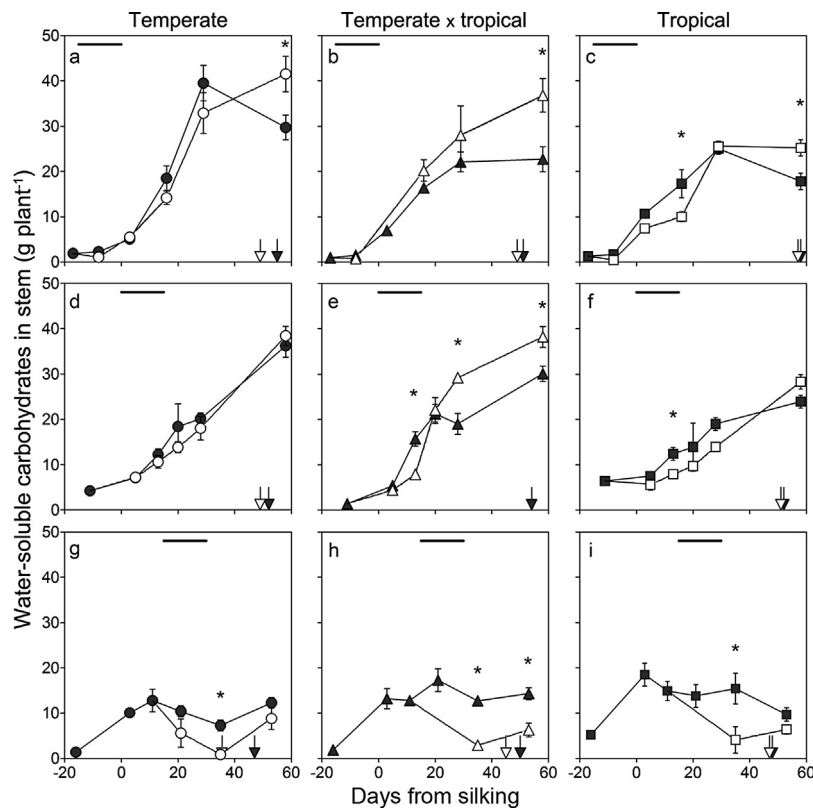


Fig. 3. Dynamics of water-soluble carbohydrates in stem of temperate (circles), temperate $\times$ tropical (triangles), and tropical (squares) hybrids exposed to heated (open symbols) and non-heated (closed symbols) conditions during three growth stages (GS₁: a–c; GS₂: d–f; GS₃: g–i) in Exp₂. Each GS covered a 15-d period (GS₁: immediately before anthesis; GS₂: from the start of silking onwards; GS₃: from the end of GS₂ onwards). Horizontal bars indicate the duration of the heating period. Open and close arrows indicate the achievement of physiological maturity of grains in heated and non-heated plots, respectively. Symbols represent the mean of each sampling date, vertical bars are the standard deviations of the mean and asterisks indicate the dates at which significant ($P < 0.05$) differences in water-soluble carbohydrates in stem were detected between temperature regime treatments.

hybrid, the only one for which (i) the condition of WSC=0 was registered at physiological maturity (Fig. 3g–i), and (ii) the black layer stage anticipated markedly in heated plots as compared to the non-heated ones (Figs. 1a–c and 3g–i).

Variation in SSR_{EGF} provided a likely ($r^2 = 0.49$, $P < 0.001$) explanation of the observed variation in WSC in stem at physiological maturity (Fig. 4). This relationship was described by a single bilinear with plateau model (Eqs. (8) and (9)) fitted to all treatment combinations. WSC availability in stem achieved maximum values when SSR_{EGF} exceeded 431 mg kernel⁻¹ (Fig. 4). Below this threshold value, plants had a sudden drop in WSC due to SSR_{EGF} reductions (-0.078 g plant⁻¹ per unit of decline in SSR_{EGF}), until carbohydrate reserves were completely depleted (i.e., WSC = 0) at $SSR_{EGF} \sim 0$ mg kernel⁻¹. Reductions in WSC in stem at physiological maturity explained a modest proportion ($r^2 = 0.32$; $P = 0.015$) of the decrease in final kernel weight (data not shown).

4. Discussion

Final kernel weight in non-heated plots was partially explained by the source-sink ratio during the critical period for kernel set (Fig. 2a), supporting previous evidences on the importance of early kernel growth (lag phase) in defining potential kernel weight in maize (Capitanio et al., 1983; Jones et al., 1996; Reddy and Daynard, 1983). However, this trend was not verified among plots heated around flowering (i.e., GS₁ and GS₂), which generally exhibited lower KW than control plots despite of very high SSR_{CP} values (Table 1). This result differed from those obtained by Gambin et al. (2008); who reported increases in final kernel weight due to enhanced SSR_{CP} of plots thinned around flowering, but no difference

in the response pattern between thinned and non-thinned control plots (i.e., a single linear model fitted their whole data set adequately). Collectively, these results suggest that the observed reduction in potential kernel weight found in current research may

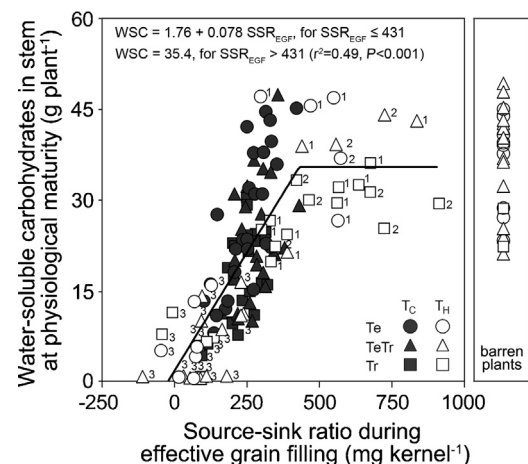


Fig. 4. Relationship between the amount of water-soluble carbohydrates (WSC) in stem at physiological maturity and the source-sink ratio during effective grain filling (SSR_{EGF}) of three maize hybrids of contrasting origin (Te: temperate; Tr: tropical; TeTr: Te $\times$ Tr) exposed to two temperature regimes (T_C: control; T_H: heated during daytime hours) during three growth stages (GS₁: 1; GS₂: 2; GS₃: 3; stage only identified for heated plots) in Exp₂. Each GS covered a 15-d period (GS₁: immediately before anthesis; GS₂: from the start of silking onwards; GS₃: from the end of GS₂ onwards). Symbols correspond to individual plants and the solid line represents the bilinear with plateau model fitted to data from heated plots only. Data from Exp₂.

be related to constraints not directly related to assimilate availability per kernel but to a direct effect of heating that has been never reported previously for this species in field conditions.

Potential kernel weight reductions due to direct effects of above-optimum temperatures have been mostly reported when the stress occurred during the lag phase of grain filling (i.e., after ovary fertilization and equivalent to GS₂ in current research), and were attributed to disruptions in endosperm cell division and amyloplast biogenesis (Commuri and Jones, 1999; Denyer et al., 1994; Engelen-Eigles et al., 2001; Hanft and Jones, 1986; Jones et al., 1985; Singletary et al., 1994). Negative effects of heating on kernel weight prior to anthesis (i.e., before ovary fertilization and equivalent to GS₁ in current research) have been less documented in cereals (Calderini et al., 1999b; Vara Prasad et al., 2008), and no evidence has been previously reported for maize. The physiological processes underlying sink limitations to final kernel weight remain unclear, but evidences in wheat (Millet, 1986), barley (Scott et al., 1983) and sorghum (Yang et al., 2009) suggest that above-optimum temperatures prior to flowering may cause physical limitations to grain growth due to their negative effects on ovary and floral structures (i.e., lemma and palea growth). These results are in agreement with evidence of reduced expansive capacity of wheat carpels exposed to high temperature before anthesis (Calderini et al., 1999a), a response that was not registered for proxy traits measured in current research (e.g., maximum water content and maximum kernel volume did not differ between contrasting thermal regime treatments imposed around flowering) and one that deserves further attention in maize.

Heat stress caused a wide variation in SSR_{EGF} that allowed us to evaluate the role of assimilate availability for grain filling in the determination of kernel weight under above-optimum temperature conditions. Heat stress during the first half of effective grain filling (GS₃) reduced SSR_{EGF} and caused the expected decrease in kernel weight (Borrás and Otegui, 2001; Cirilo and Andrade, 1996; Maddonni et al., 1998). However, the observed response of kernel weight to changes in SSR_{EGF} differed in some aspects from those usually reported in maize (op. cit.). A single bilinear with plateau model did not fit the whole data set as expected, because kernel weight values had positive (non-heated plots) or negative (heated plots) bias depending upon thermal regime. An independent model could be fitted only for heated plots (Fig. 2b), as data from the non-heated ones were clustered with no clear trend within narrow ranges of SSR_{EGF} and kernel weights as compared to their heated counterparts. Kernel weight values in non-heated plots had a positive bias respect to the model fitted to heated plots in spite of the large SSR_{EGF} values registered among heated plots, particularly for GS₁ and GS₂ treatments. These effects became more notorious when the analysis was based on the change relative to control plots of both SSR_{EGF} and kernel weight (Fig. 5). Within the quantitative framework proposed by Borrás et al. (2004); results from current research matched the expected trend for reduced (GS₃) but not for enhanced SSR_{EGF} (GS₁ and GS₂). For the former, most cases had a negative bias respect to the 0-change in relative kernel weight and matched the fit to mean values of Borrás et al. (2004). For the latter, almost all data were below the fit representative of the 10% less responsive cases of these authors. This response to heating during GS₁ and GS₂ is an additional evidence of permanent and source-independent heat effects on potential kernel weight, which cannot be compensated by enhanced source availability during active grain filling.

As has been shown for other stresses, decreases in SSR_{EGF} reduced kernel weight by shortening grain-filling duration (Egharevba et al., 1976; Jones and Simmons, 1983), whereas increments in SSR_{EGF} had no effects on this trait (Andrade and Ferreiro, 1996; Schoper et al., 1982). Duration of effective grain filling was also associated with the rate of water loss from kernels after reaching maximum water content, which was maximum for the Te hybrid heated during GS₃.

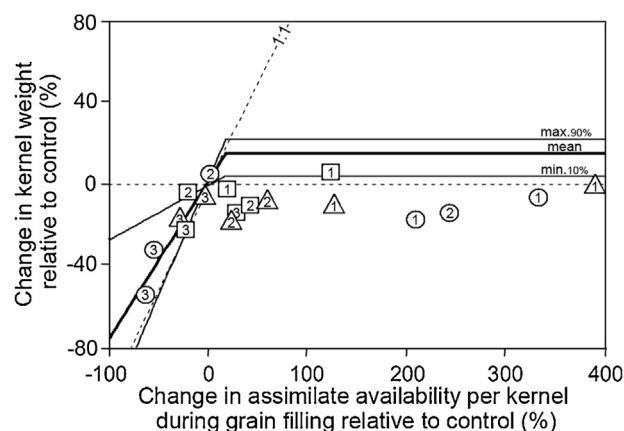


Fig. 5. Relationship between the change in kernel weight and the change in assimilate availability per kernel during grain filling (as percent of controls) of temperate (circles), temperate × tropical (triangles), and tropical (squares) hybrids exposed to heat stress during three growth stages (GS₁: 1; GS₂: 2; GS₃: 3) in two experimental years. Each GS covered a 15-d period (GS₁: immediately before anthesis; GS₂: from the start of silking onwards; GS₃: from the end of GS₂ onwards). Symbols correspond to the mean values of each experimental year. Dashed lines represent the theoretical limitations produced exclusively by the source (1:1 ratio line) and the sink (horizontal line) of assimilates during grain filling. Solid lines indicate maximum (max. 90%), mean, and minimum (min. 10%) responses of kernel weight to changes in assimilate availability per kernel during grain filling estimated by Borrás et al. (2004).

This fact, together with the null effect of the stress on kernel growth rate, enhanced kernel desiccation rate but also kernel moisture concentration at physiological maturity, which is consistent with previous studies in maize (Sala et al., 2007a,b; Westgate, 1994) and other grain crops (Barlow et al., 1980; Brooks et al., 1982; Gooding et al., 2003). Additionally, heating during GS₃ reduced maximum kernel volume due to the combination of two factors. First, maximum kernel volume was reached close to physiological maturity (Gambín et al., 2007), and not at the time of maximum kernel water content (Saini and Westgate, 2000). Therefore, in those cases in which a pronounced shortening of grain-filling duration occurred, maximum kernel volume was reduced. Second, as already mentioned, heating caused an increased rate of water loss from kernels prior to reaching the maximum kernel volume that may have reduced kernel volume expansion. However, small reductions in kernel weight due to heating around flowering could not be associated with changes in kernel water content (Borrás and Westgate, 2006) or kernel growth rate (Borrás and Otegui, 2001) because significant differences could not be detected for the dynamics of water and biomass accumulation in grains between control and heated plots (Table 1).

The pattern of WSC in stem evaluated during Exp₂ provided valuable information that strengthened the established association between assimilate availability for grain filling and kernel growth. Heat stress altered the dynamics of WSC in stem by affecting the assimilate availability per kernel during effective grain filling. On one hand, SSR_{EGF} reductions due to heating during GS₃ caused a more rapid depletion of carbohydrate reserves in stem, which is in agreement with previous studies in maize that reduced the amount of assimilates for grain filling through defoliation (Jones and Simmons, 1983; Sayre et al., 1931), shading (Andrade and Ferreiro, 1996; Uhart and Andrade, 1995) and drought (Ouattar et al., 1987b; Westgate and Boyer, 1985) during active kernels growth. On the other hand, the positive effect of heating around flowering on SSR_{EGF} promoted the deposition of reserves in stem, as was observed in experiments that involved manipulative treatments aimed to increase assimilate availability per kernel during effective grain filling, such as restricted pollinations (Hume and Campbell, 1972; Sayre et al., 1931), shading

around flowering (Uhart and Andrade, 1995), ear removal (Christensen et al., 1981; van Reen and Singleton, 1952), kernels removal (Jones and Simmons, 1983), and thinning the stand during effective grain filling (Andrade and Ferreiro, 1996).

Study of WSC allowed us to elucidate the cause of the high sensitivity of the Te hybrid grain yield to heating during the first half of effective grain filling reported in a previous study (Rattalino Edreira and Otegui, 2012). Our results showed that the pattern of WSC in stem was similar among genotypes within each sowing date, so that the availability of carbohydrate reserves prior to the stress did not vary much among them (Fig. 3). However, the Te hybrid experienced the earliest depletion of carbohydrate reserves due to heating during GS₃ (Fig. 3g). This response could be related to a reduced supply of assimilates from current photosynthesis during effective grain filling in this hybrid as compare to the other genotypes, an expected consequence of its decreased radiation use efficiency when exposed to heat stress (Rattalino Edreira and Otegui, 2012).

Described variations in the amount of WSC accumulated in the stem at physiological maturity were associated with the assimilate availability per kernel during effective grain filling. The bilinear with plateau model fitted to this relationship (Fig. 4) suggested a high contribution of carbohydrate reserves to grain filling when SSR_{EGF} values were low, although this source of assimilates was insufficient to avoid the arrest in kernel growth observed in some cases. This high sensitivity of maize kernel to photoassimilate production during grain filling may be due to the fact that carbohydrate reserves are already part of the source required for filling the grain in most normal growing conditions (Andrade and Ferreiro, 1996; Jones and Simmons, 1983; Uhart and Andrade, 1995; Kiniry and Otegui, 2000). Therefore, any constraint on photosynthetic activity that reduces assimilate availability during this phase cannot be fully compensated by the remobilization of reserves. Additionally, the bilinear model was able to describe the opposite response when plants exhibited high SSR_{EGF} values (i.e., plants heated around flowering). Such plants had WSC values as large and variable as those registered for barren plants, which indicates that these plants reached their maximum storage capacity in the stem.

Finally, we want to call attention on the fact that experiments were performed in field conditions for producing a more realistic assessment of heat stress effects on the physiological determinants of maize kernel weight. However, the technique used to impose heat stress might have introduced some uncertainty in final results due to changes in CO₂ and VPD. Daytime values registered in the ad-hoc plots were (i) larger among T_C plots (always >300 ppm) than among T_H plots (always >225 ppm) for CO₂, and (ii) always lower among T_H plots (0.019–1.386 kPa) than among T_C plots (1.115–1.928 kPa) for VPD. Differences in CO₂ values between temperature regimes could have an effect on maize photosynthesis (e.g., a decrease in T_H plots), but this trend could be mitigated by an increase in stomatal conductance in response to reduced CO₂ (Ainsworth and Rogers, 2007). Similarly, the described variation in VPD values could affect photosynthesis due to its effect on stomatal conductance (Gholipour et al., 2010; Yang et al., 2012). Control shelters had higher midday VPD values than those of heated shelters. Thus, any eventual VPD effect on stomatal conductance would probably have reduced the effects of heat stress among T_H plots. All these evidences suggest that observed responses in the current paper had a dominant effect of temperature rather than of CO₂ and VPD.

5. Conclusions

Heat stress reduced final kernel weight across all studied periods. Heating around flowering enhanced the assimilate availability per kernel during both the critical period for kernel

set and the effective grain-filling period, but slight reductions in final kernel weight were observed. This effect may be attributable to direct negative effects of above-optimum temperatures on potential kernel weight. Heating during the first half of effective grain filling reduced the assimilate availability per kernel during effective grain filling. This effect caused an earlier cessation of grain filling, particularly in the temperate hybrid. This response was accompanied by increases in the depletion of carbohydrate reserves of the stem and in the rate of water loss from kernels after reaching maximum water content. Therefore, robust associations could be established between (i) WSC in stem and SSR_{EGF} and (ii) the rate of water loss from kernels and the duration of effective grain filling. There were genotypic differences among hybrids of contrasting origin in the response of kernel weight to heating. Results indicated that the enhanced sensitivity to this constraint of the temperate hybrid seemed linked to the earlier cessation of grain filling, which matched the occurrence of complete depletion of reserves in stems.

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