RESEARCH

Heterotic Response for Grain Yield and Ecophysiological Related Traits to Nitrogen Availability in Maize

E. M. Munaro,* K. E. D'Andrea, M. E. Otegui, A. G. Cirilo, and G. H. Eyhérabide

ABSTRACT

Maize (*Zea mays* L.) hybrid vigor for plant grain yield (PGY) is associated with heterosis for plant biomass at maturity (aboveground biomass at physiological maturity [$Biomass_{PM}$]), kernel number per plant (KNP), and harvest index (HI); however, no evidence of the effects of nitrogen (N) availability or combination of abiotic stresses on heterosis for physiological components of PGY has been reported. The objective of this study was to determine the response of heterosis for ecophysiological traits related to PGY at contrasting N supply levels in a set of six inbred lines and 12 derived hybrids. Field experiments were conducted in five growing seasons at low nitrogen (LN; no N added) and high nitrogen (HN) supply (200 or 400 kg N ha^{-1}) under irrigation and dryland farming. Increased PGY (65% for hybrids and 30% for inbreds) was ascribed to similar increase in $Biomass_{PM}$ as no increase in HI was found. Heterosis for PGY was higher under HN (137%) than LN (87%). A similar response was observed for traits related to light capture and biomass accumulation. Heterosis for HI did not differ between HN (31%) and LN (28%). Heterosis for PGY was associated ($p < 0.01$) with heterosis for KNP, $Biomass_{PM}$, radiation use efficiency (RUE) during grain filling, HI, and traits related to maximum light capture. Heterosis for PGY at LN was also correlated with heterosis for RUE at critical period (i.e., 30 d bracketing silking) and kernel weight. Under the combined effect of N and drought, PGY heterosis was reduced and more affected at HN (59%) than at LN (70%).

E.M. Munaro, K.E. D'Andrea, and M.E. Otegui, Dpto. de Producción Vegetal, Facultad de Agronomía, Universidad de Buenos Aires, Av. San Martín 4453 (C1417DSE), Ciudad de Buenos Aires, Argentina; K.E. D'Andrea, Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET); M.E. Otegui, Instituto de Investigaciones Fisiológicas y Ecológicas Vinculadas a la Agricultura (IFEVA-CONICET); G.H. Eyhérabide and A.G. Cirilo, Instituto Nacional de Tecnología Agropecuaria (INTA), Estación Experimental Agropecuaria Pergamino, Buenos Aires, Pergamino, Argentina. Received 8 Aug. 2010. *Corresponding author (munaro@agro.uba.ar).

Abbreviations: ASI, anthesis-silking interval; $Biomass_{PM}$, aboveground biomass at physiological maturity; $Biomass_{R2}$, aboveground biomass at R_2; $Biomass_{V14}$, aboveground biomass at V_{14}; E_1, apical ear; EGR_{CP}, apical ear growth rate during the critical period; fIPAR, fraction of incident photosynthetically active radiation intercepted by canopy; $fIPAR_{MAX}$, maximum fraction of incident photosynthetically active radiation intercepted by canopy; $fIPAR_{PM}$, fraction of incident photosynthetically active radiation intercepted by canopy at physiological maturity; G, genotype; HI, harvest index; HN, high nitrogen; IPAR, incident photosynthetically active radiation; IPARi, intercepted incident photosynthetically active radiation; $IPARi_{CP}$, intercepted incident photosynthetically active radiation during the critical period; $IPARi_{GF}$, intercepted incident photosynthetically active radiation during the grain-filling period; $IPARi_{PM}$, intercepted incident photosynthetically active radiation during the whole cycle; KNE_1, kernel number per apical ear; KNP, kernel number per plant; KW, kernel weight; LAI, leaf area index; LAI_{MAX}, maximum leaf area index; LAI_{PM}, leaf area index at physiological maturity; LN, low nitrogen; MPH, midparent heterosis; PAR, photosynthetically active radiation; PC, principal component; PGR_{CP}, plant growth rate during the critical period; PGY, plant grain yield; PPFD, photosynthetic photon flux density; RUE, radiation use efficiency; RUE_{GF}, radiation use efficiency during the grain-filling period; RUE_{PM}, radiation use efficiency at physiological maturity; RUE_{V14}, radiation use efficiency up to V_{14}; Y, year.

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Heterosis or hybrid vigor (i.e., superior performance of F_1 hybrid progeny relative to the parental phenotypes) in maize (*Zea mays* L.) is most noticeable as an increase in plant size (East and Jones, 1919); however, its main effect is an additional production of seed (Troyer and Weillin, 2009). Maize exhibits heterosis for a wide range of traits, but its magnitude is highly variable depending on the choice of parents and the trait measured (Springer and Stupar, 2007). Studies regarding heterosis have focused on morphological traits measured at a single point in time (e.g., at flowering or at harvest), such as plant height, ear height, percent lodging, etc. (Duvick 2005, Flint-Garcia et al. 2009). These traits can be described as "static" and do not necessarily have a functional relationship with grain yield because of their non-process-related nature. This constraint is common to many reports in which heterosis for morphological traits of easy measurement or numeric components of yield are considered. In a physiological interpretation of hybrid vigor from a functional approach, Tollenaar et al. (2004) determined that heterosis for source components affecting the rate of dry matter accumulation (i.e., maximum leaf area index [LAI_{MAX}], staygreen, and sustained photosynthesis of green leaf area during the grain-filling period) were highly associated with the expression of heterosis for grain yield in maize grown under optimum conditions. Heterosis for grain yield was also associated with heterosis for harvest index (HI) resulting from a differential partitioning of biomass to grain between inbreds and hybrids during the critical period (Echarte and Tollenaar, 2006; D'Andrea et al. 2009). In various species heterosis for grain yield is strongly associated with heterosis for grain number (Leng, 1954; Blum, 1970; Echarte and Tollenaar, 2006), therefore, a same pattern of heterotic association would be expected when analyzing physiological components determining grain yield (i.e., light capture, biomass production, and partitioning to reproductive structures during critical periods). Studies that include heterosis for the abovementioned physiological traits are currently lacking.

Heterosis generally varies with environmental conditions and its contribution to stress tolerance is relatively enhanced under drought (Betran et al., 2003), high plant density (Liu and Tollenaar, 2009b), or excessive soil moisture stress (Zaidi et al., 2007), particularly for grain yield. More variable results have been obtained for growth rate. To this extent Echarte and Tollenaar (2006), when studying kernel set in two maize hybrids and three inbred lines grown under water stress and high plant density, did not find plant growth rate during the critical period (PGR_{CP}; i.e., during a 30 d period centered at silking) to be consistently greater in hybrids than in their parental inbred lines. When analyzing nitrogen (N) stress and a large set of genotypes (G), D'Andrea et al. (2009) found PGR_{CP} to be consistently larger for hybrids than inbreds, whereas apical ear growth rate during the critical period (EGR_{CP}) did not differ between these two groups of genotypes. Apparently, drought stress has a greater effect on the expression of inbreeding depression (the reverse manifestation of hybrid vigor; Falconer and Mackay, 1996) than N stress (Betran et al., 2003; D'Andrea et al., 2009). Changes in heterosis with stress (high plant density, low nutrient availability, drought, excessive soil moisture, etc.) may therefore depend to some extent on the type of trait and its association with fitness, as well as the nature of the environmental stress (Hoffmann and Parsons, 1991). Moreover, environmental variability may affect the relationships of physiological components of heterosis for grain yield depending on the main effect of the stress (e.g., tissue expansion, photosynthesis, or biomass partitioning). The only evidence of the effects of stress on the response of heterosis for physiological traits in maize comes from research conducted recently in Canada with only two inbred lines and their corresponding hybrid cropped at high plant density (Liu and Tollenaar, 2009b) or shading stress during three phases of development (Liu and Tollenaar, 2009a). These studies, however, are limited in their informative value in that they employed few genotypes. Higher heterosis for grain yield was observed under both stressful conditions (i.e., high plant density or imposed shading), although differences were found regarding the physiological traits associated with heterosis for grain yield. When stress was originated by increasing plant density, an increase in heterosis was observed only for HI, whereas stress originated by shade increased heterosis for aboveground dry matter accumulation as well. Heterosis for grain yield, therefore, is a variable trait that depends on environmental conditions that affect inbreds and hybrids differently. The understanding of the effects of abiotic stress on physiological processes underlying the expression of heterosis remains unclear, in particular to what extent heterosis can be affected by low N stress in maize. Studies regarding the response of heterosis across environmental conditions (i.e., years and resource availability) would provide a better knowledge of the differential responses of heterosis for physiological traits to specific environments, improving the efficiency with which the breeder can characterize material (i.e., phenotyping).

This work provides an analysis of the differences in response pattern between inbreds and their derived hybrids in attributes related to light capture, biomass production, and grain yield and its components under contrasting soil N levels during 5 yr and for a representative number of progenitors (six) and F_1 crosses (12). The objectives of this study were (i) to determine the level of heterosis for ecophysiological traits related to grain yield under high nitrogen (HN) and low nitrogen (LN) environments and (ii) to study the ecophysiological processes determining heterosis for grain yield across environments (i.e., weather conditions), analyzing the pattern and strength of heterotic associations between grain yield and its physiological components.

MATERIALS AND METHODS

Genetic Material

Twelve single-cross maize hybrids (six direct crosses and their reciprocals) were selected from all possible crosses of six inbred lines (B100, ZN6, LP662, LP611, LP561, and LP2). Lines were previously phenotyped by D'Andrea et al. (2006, 2009) and presented variability in breeding eras, origin, canopy size, grain yield, and grain yield components. Inbreds also differed in the heterotic group of origin; B100 is a U.S. semident germplasm (Hallauer et al., 1995) and the rest of the inbreds belong to Argentine flint germplasm. Additionally, inbreds LP2 and LP561 were derived from Caribbean germplasm. Hybrids included in this study were B100 × LP2, B100 × ZN6, B100 × LP561, ZN6 × LP561, ZN6 × LP611, LP561 × LP662, and all reciprocal crosses. For details on the method used for hybrid development refer to D'Andrea et al. (2009).

Crop Husbandry and Experimental Design

Field experiments were conducted at the National Institute of Agricultural Technology (INTA) Pergamino Experimental Station, Argentina (33°56' S, 60°34' W) on a Typic Argiudoll soil during 2002–2003 (Exp. 1), 2003–2004 (Exp. 2), 2004–2005 (Exp. 3), 2006–2007 (Exp. 4 and Exp. 5), and 2008–2009 (Exp. 6 and Exp. 7). The top soil (0–40 cm layer) had an organic matter content of 22 (Exp. 1 and Exp. 2), 14 (Exp. 3), 24 (Exp. 4), 15 (Exp. 5 and Exp. 7), and 50 g kg^{-1} (Exp. 6). No P fertilizer was needed. Inorganic N at sowing was 55, 23, 40, 34, 29, 87, and 62 g kg^{-1} (Exp. 1 to Exp. 7, respectively). For Exp. 1, 2, 3, 4, and 6 supplemental irrigation was given to prevent water stress by keeping the uppermost 1 m of soil near field capacity, whereas experiments 5 and 7 were conducted under dryland farming.

Maize was hand planted on 1 November, 9 October, 8 November, 1 November, 20 October, 26 October, and 23 October (Exp. 1 to Exp. 7, respectively). Treatments were a factorial combination of genotypes and two N levels. These levels were a control with no added N (LN) and a HN condition that was fertilized with 400 (Exp. 1 to Exp. 3) and 200 kg N ha^{-1} (Exp. 4 to Exp. 7), supplied as urea in two applications between sowing and at the 12-ligulated leaf stage (V_{12}; Ritchie et al., 2008). The fertilizer was mechanically incorporated into the soil shortly after application. The experimental design was a split plot with main plots organized in randomized complete blocks, with N availability in the main plot, genotypes in the subplot (hereafter termed plots), and three replicates. Two guard rows of a short season commercial hybrid were planted on each side of the experiments. Each plot consisted of three rows of 5.5 m length with a spacing of 0.7 m between the rows. Plots were hand planted at a rate of three seeds per hill and thinned to one plant per site at V_3. Final plant population was always 7 plants m^{-2}. Weeds and insects were controlled throughout the growing season. Hourly recorded values of incident solar radiation and air temperature were obtained at the experimental site with a LI-COR 1200 (LI-COR, Lincoln, NE) weather station. Rainfall events were also registered in situ on a daily basis. Daily incident solar radiation was converted into incident photosynthetically active radiation (IPAR) by multiplying by 0.45 (Monteith, 1965), and accumulated thermal time (TT; in °C d^{-1} with base temperature of 8°C) was computed from mean daily air temperatures from sowing onward as proposed by Ritchie and NeSmith (1991). Initial soil water (in percent of maximum transpirable soil water) was obtained from estimates produced by the national meteorological service (Servicio Meteorológico Nacional, 2010).

Measurements

Five successive plants were tagged at V_3 on the central row of each plot to follow leaf appearance, senescence dynamics, and flowering events. Tags were placed at identified positions along the stem, which allowed the identification of individual leaves. The numbers of ligulated and senesced (more than half of leaf blade yellowed) leaves per plant were registered weekly between seedling emergence and physiological maturity on all tagged plants. Individual leaf area was estimated at silking as lamina length × maximum width × 0.75 (Montgomery, 1911), and maximum plant leaf area obtained at this stage by summing for all green leaves measured on each plant. Maximum leaf area index (LAI_{MAX}) was calculated as the product of leaf area per plant and number of plants per unit land. The fraction of IPAR intercepted by the canopy (fIPAR) was estimated fortnightly from V_5 onward as in Eq. 1.

$$fIPAR = 100\,[1 - (I_b/I_a)], \quad [1]$$

in which I_b is the incident photosynthetic photon flux density (PPFD) below the bottommost green leaves and I_a is the incident PPFD at the top of the canopy (Gallo and Daughtry, 1986). Measurements were made with a line quantum sensor (Cavabar, Cavadevices, Argentina) at a rate of three determinations per plot for I_b and one each five plots for I_a. Daily fIPAR values were obtained by linear interpolation between successive measurements. The intercepted incident photosynthetically active radiation (IPARi) was computed as the product between fIPAR and IPAR, and cumulated values obtained for (i) the critical period for kernel set between approximately V_{14} and R_2 (i.e., intercepted incident photosynthetically active radiation during the critical period [$IPARi_{CP}$]), (ii) the grain-filling period between R_2 and physiological maturity (i.e., intercepted incident photosynthetically active radiation during the grain-filling period [$IPARi_{GF}$]), and (iii) the whole cycle (i.e., intercepted incident photosynthetically active radiation during the whole cycle [$IPARi_{PM}$]).

Anthesis date (i.e., at least one extruded anther visible at the tassel) and apical ear (E_1) silking date (i.e., at least one silk visible after extruded from the husks) were registered for each tagged plant. The anthesis-silking interval (ASI) and mean dates of anthesis and silking were computed for each plot as the average of individual plant values (Uribelarrea et al., 2002). Thermal time to anthesis and silking were determined for each entry.

Biomass production was estimated at V_{14} (aboveground biomass at V_{14} [$Biomass_{V14}$]) and at R_2 (aboveground biomass at R_2 [$Biomass_{R2}$]) by means of allometric models. This is a widely tested, well documented approach (Vega et al., 2000; Borrás and Otegui, 2001; Maddonni and Otegui, 2004) that has been applied to hybrids and inbreds growing under different abiotic stress conditions (D'Andrea et al., 2006; Echarte and Tollenaar, 2006; D'Andrea et al., 2009). All relationships were highly significant ($p < 0.001$), and no difference was detected in model parameters between reciprocal hybrids (D'Andrea et al., 2009). Mean values of plant growth rate (PGR_{CP}; in g d^{-1}) and ear growth rate (EGR_{CP}; in g d^{-1}) during the critical period for kernel set (i.e., between the

start of active ear growth at approximately V_{14} and the start of active grain filling at R_2; Westgate et al., 2004) were computed, and biomass partitioning ratio around silking was obtained for each treatment combination as the quotient between mean EGR_{CP} and mean PGR_{CP}. Radiation use efficiency (RUE) was estimated as the quotient between biomass production and cumulative IPARi for different periods along the cycle: (i) up to V_{14} (i.e., radiation use efficiency up to V_{14} [RUE_{V14}]), as representative of predominant vegetative growth, (ii) for the critical period for kernel set between approximately V_{14} and R_2 (i.e., RUE_{CP}), (iii) for the grain-filling period between R_2 and physiological maturity (i.e., radiation use efficiency during the grain-filling period [RUE_{GF}]), and (iv) at physiological maturity (i.e., radiation use efficiency at physiological maturity [RUE_{PM}]), as representative of the whole cycle. All tagged plants were individually harvested at physiological maturity, and prolificacy was computed as the number of grained ears per plant. Plant material was oven dried at 60°C for 7 d and weighed for final shoot plant biomass determination (aboveground biomass at physiological maturity [$Biomass_{PM}$]). Each grained ear was individually hand shelled, and kernel number was counted. Kernel number per plant (KNP) was calculated by adding the kernels counted in E_1 (kernel number per apical ear [KNE_1]) and subapical ear (when present). Grain yield was computed for each harvested plant (plant grain yield [PGY]), and individual kernel weight (KW) was obtained as the quotient between PGY and KNP. For each treatment combination we computed mean values of (i) harvest index (HI), as the ratio between PGY and $Biomass_{PM}$, (ii) plant reproductive capacity, as the ratio between KNP and PGR_{CP}, and (iii) apical ear reproductive capacity, as the ratio between KNE_1 and EGR_{CP}.

Statistical Analysis

Preliminary ANOVA was performed for each experiment. Bartlett's test was employed in testing ($p < 0.05$) for homogeneity of variances followed by Levy's procedure for multiple comparisons among variances. Experiments were grouped accordingly. Experiments 1 through 4 (irrigated) and Exp. 5 (rainfed) had equal variance ($p > 0.05$).The experiments that were left out of the combined ANOVA were those conducted during the 2008–2009 growing season (i.e., Exp. 6 and Exp. 7, irrigated and rainfed, respectively). Analysis of variance across experiments showing N treatment effect was conducted by means of PROC GLM procedure of SAS v. 8.2 (SAS Institute, 1999). Nitrogen treatments and genotypes were considered as fixed effect, and experiments and replications within experiments treated as random effects. When main or interaction effects were significant ($p < 0.05$), a *t* test was used for comparisons among means.

Assuming absence of significant reciprocal effects (D'Andrea et al., 2009), heterosis was calculated for each experiment by pooling values of direct and reciprocal crosses. Midparent heterosis (MPH), that is, the superiority of a hybrid compared to the parental mean, was calculated for each trait as in Eq. 2.

$$MPH = ([F_1 - MP)/MP] \times 100, \quad [2]$$

in which F_1 is the mean of the direct and reciprocal single-cross hybrids, and MP is the midparental value. Statistical significance of heterosis values for each trait and its comparisons between N treatments were determined by *t* tests.

Pearson's correlation coefficients (*r*) under LN and HN conditions were computed, using Infogen (Balzarini and Di Rienzo, 2004), to determine the relationships between heterosis for PGY and heterosis for ecophysiological traits. Comparison of correlation coefficients was done using Fisher's Z transformation. Principal component analysis (PCA) was performed on the genotype × trait heterosis matrix containing standardized data. A biplot was constructed for LN and HN using the first two principal components (PC1 and PC2) to determine the relationships between heterosis for PGY, ecophysiological related traits, and genotypes. In the biplots, genotypes are represented by points and traits by vectors from the origin, and can be interpreted as follows. Genotypes that are close together are similar in their specific responses for all traits analyzed. For any particular trait, genotypes can be compared by projecting a perpendicular from each genotype point to the trait vector, that is, entries that are further along in the positive direction of a trait vector show higher relative responses for this trait and vice versa. The biplots also display the strength of the associations among traits in terms of their genotypic relative responses. Acute angles between any two trait vectors indicate positive associations, that is, they are positively correlated; 90° angles indicate no association; and angles greater than 90° indicate negative associations (Chapman et al., 1997; Kroonenberg, 1997).

RESULTS

Weather Conditions

The meteorological conditions varied widely between experimental years (Y; Fig. 1). In general, mean incident solar radiation was similar for the 2002–2003, 2003–2004, 2006–2007, and 2008–2009 seasons (23.2, 23.9, 22.9, and 24.1 MJ m^{-2} d^{-1} respectively), but records during 2004–2005 indicated a 35% reduction (17.5 MJ m^{-2} d^{-1}) that became more pronounced (47%) during the grain-filling period in which the mean incident solar radiation was 15.1 MJ m^{-2} d^{-1} (Fig. 1).The amount and distribution of precipitation during the growing season differed between experimental years. During the 2008–2009 summer season a severe drought took place in the Pampas region, occurring from 3 wk before silking to the end of the grain-filling period. The 2008–2009 growing season had the lowest amount of rainfall (204 mm), followed by the 2003–2004 season with 351 mm, but the latter season had a better distribution in relation to timing of flowering (Fig. 1). The highest amount of rainfall during the maize growing season recorded in this study was 676 mm in 2006–2007. Therefore, a large variation in water supply was recorded during the growing seasons in which the dryland experiments were conducted (2006–2007 and 2008–2009). The amount of available soil water stored at planting differed between these two seasons (100 and 25% of transpirable soil water for the former and the latter, respectively). As a result of low precipitation observed during the 2008–2009 fallow period, residual nitrate levels before planting were above the recommended threshold (i.e., 50 g kg^{-1}) for N fertilization. These differences in initial soil N among growing seasons affected the overall response to N supply across experiments. Moreover, the high level of

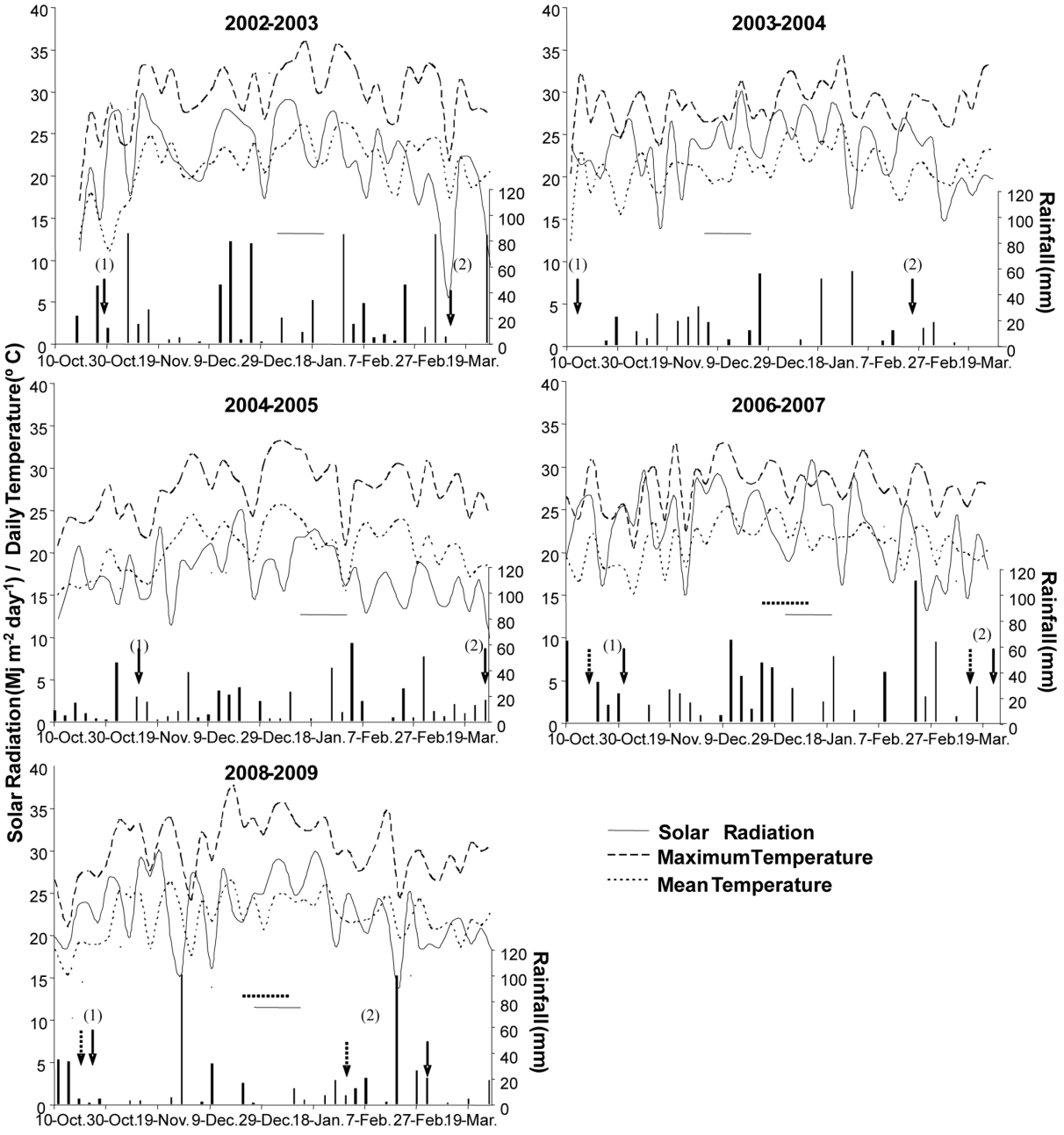

Figure 1. Daily values of maximum and mean temperatures, solar radiation, and rainfall for five experimental seasons. Arrows indicate timing of planting date (1) and timing of physiological maturity (2). Dashed and solid horizontal lines represent mean flowering period for dryland and irrigated experiments, respectively.

soil organic matter measured in Exp. 6, combined with high mean temperatures and an adequate water supply, may have resulted in high soil N mineralization observed from the onset of V_{14} stage (Di Nápoli and Maddonni, 1996), masking differences between N treatments for this experiment. The difference in precipitation between 2006–2007 and 2007–2008 and 2008–2009 growing seasons was more remarkable during the critical period, in which the latter had a 78% reduction in rainfall compared to the former seasons and a 68% reduction when compared to historical records (1910–2007).

Experimental years did not differ in overall mean and mean maximum air temperatures (20.7 to 21°C and 27.3 to 28.1°C, respectively), except for the 2002–2003 and 2008–2009 seasons that had higher overall mean (22.5 and 24°C, respectively) and mean maximum values (30.6 and 32°C, respectively). These differences intensified during the reproductive stage, mainly because the 2002–2003 and 2008–2009 seasons had maximum records above 35°C during 30 and 40% of the critical period, respectively. The remaining seasons did not experience these conditions for the period bracketing silking. In addition, the 2008–2009 season had higher maximum temperatures throughout the grain-filling period that anticipated physiological maturity in the dryland experiment compared to the irrigated one (Fig. 1). The warm and dry conditions in 2008–2009 contributed to the lowest observed values for grain yield in this study.

The above-mentioned variability in weather conditions between experimental years caused a significant G × N × Y interaction for most measured traits. The effects of N availability on the expression of heterosis is the principal focus in the following sections, therefore the experiments were grouped (as shown in Table 1 and accordingly to results of Bartlett's and Levy's tests mentioned previously) to evaluate consistency in response to LN stress and avoid confounding climatic conditions (heat and extreme drought stress) which may obscure interpretation of results. The experiments that were left out of the combined ANOVA were those conducted during the 2008–2009 growing season (i.e., Exp. 6 and Exp. 7, irrigated and rainfed, respectively). Therefore the group showing predominantly N effect comprised Exp. 1 through 4 (irrigated) and Exp. 5 (rainfed).

Phenology

Inbred lines had higher thermal requirements to flowering than hybrids; however, the same response pattern to LN stress was observed regardless of genotype group. Low N treatment caused a slight increase in thermal requirements, which was significant ($p < 0.05$) only for thermal time to silking in hybrids (Table 1). Though significant ($p < 0.01$), mean midparent heterosis for these traits was negative (i.e., the preflowering period was reduced for the F_1 progeny as compared to the parental inbreds), small, and similar ($p < 0.05$) between N treatments for Exp.1 through 5 (Table 2).

The ASI was in general larger in inbreds than hybrids. Low N treatment did not significantly affect ASI in inbreds, whereas hybrids had a shorter ASI under HN supply (Table 1). No significant heterosis was recorded for ASI across all experiments; however, additional drought stress reduced ($p < 0.01$) hybrid advantage for this trait.

Canopy Size and Light Capture

Traits related to light capture (LAI_{MAX}, leaf area index at physiological maturity [LAI_{PM}], maximum fraction of incident photosynthetically active radiation intercepted by canopy [$fIPAR_{MAX}$], fraction of incident photosynthetically active radiation intercepted by canopy at physiological maturity [$fIPAR_{PM}$], $IPARi_{CP}$, $IPARi_{GF}$, and $IPARi_{PM}$) were greater in hybrids than in inbreds. In experiments with significant N effect (i.e., Exp. 1 to Exp. 5), LN reduced ($p < 0.001$) all these traits in both inbreds and hybrids (Table 1), but the magnitude of the response was usually larger for the latter than for the former.

Under LN alone, mean heterosis for LAI_{MAX} and LAI_{PM} decreased from HN to LN treatment (from 51% at HN to 32% at LN for LAI_{MAX} and from 134% at HN to 23% at LN for LAI_{PM}; Table 2). The same response pattern was found for heterosis for $fIPAR_{PM}$; however, hybrid advantage for light capture at the end of the cycle was not significant at LN. In contrast, heterosis for $fIPAR_{MAX}$ was significant ($p < 0.001$) at both N conditions and similar values were found (28% for HN and 31% for LN). Heterosis for IPARi during the critical period ($IPARi_{CP}$) was similar for HN and LN treatments (32 and 31%, respectively; Table 2), that is, hybrids and inbreds did not differ in their sensitivity to LN during this period (15 and 16% reduction for hybrids and inbreds, respectively; Table 1).The main difference in response to LN for light capture between the two groups of genotypes was for $fIPAR_{PM}$, $IPARi_{GF}$, and total cumulated $IPARi_{PM}$, for which the negative effect of reduced N supply was larger on hybrids than on inbreds (Table 1) and caused a decline in heterosis for these traits at LN (Table 2).

When an additional stress occurred, hybrid advantage for LAI_{MAX}, $fIPAR_{MAX}$, $IPARi_{GF}$, and $IPARi_{PM}$ was still significant ($p < 0.001$), but changes in magnitude of heterosis occurred. The additional stress masked differences in mean heterosis between N treatments (Table 2). In contrast, mean heterosis for LAI_{PM} and $fIPAR_{PM}$ varied both in magnitude and significance between N treatments with an additional drought stress (e.g., for LAI_{PM}, 33% and $p < 0.05$ at HN, −2% and $p > 0.05$ at LN).

Biomass Production and Allocation

Under LN alone, hybrids showed a higher efficiency than inbreds for converting intercepted photosynthetically active radiation (PAR) into dry matter (i.e., RUE), and this advantage increased as conditions for high RUE improved (e.g., HN). A similar trend was found for biomass production along the whole cycle, PGR_{CP} and EGR_{CP}. The partitioning ratios (HI and $EGR_{CP}\ PGR_{CP}^{-1}$) were higher for hybrids compared to inbreds; however, these ratios were not significantly reduced by LN in hybrids nor inbred lines (Table 3). Low N significantly ($p < 0.05$) reduced (i) biomass production, PGR_{CP}, and EGR_{CP} in hybrids and inbreds (average across mentioned traits of −30 and −18% for hybrids and inbreds, respectively) and (ii) RUEs only in hybrids (on average −20%). In contrast, HI and $EGR_{CP}\ PGR_{CP}^{-1}$ ratio were not significantly reduced by low N supply.

In experiments with significant N effects, mean heterosis at HN was significant ($p < 0.05$) for some RUE values (Table 2) and EGR_{CP} (48%) but not under LN (Table 2). As mentioned above, in both genotype groups there was, in general, an increase in biomass accumulation with increasing N availability, but hybrids were more responsive than parental inbred lines. Therefore, mean midparent heterosis for biomass accumulation was always moderate for LN (on average 50%) and high for HN (on average 80%) with the highest values corresponding to $Biomass_{V14}$. The difference in heterosis between N treatments increased with the advance in crop cycle (Table 2); that is, heterosis for $Biomass_{V14}$ and $Biomass_{PM}$ at LN was 73 and 56% of that at HN, respectively. A similar pattern was observed for HI and PGR_{CP} that were significant regardless of N condition. Contrarily, mean heterosis for biomass partitioning ratio ($EGR_{CP}\ PGR_{CP}^{-1}$) was significantly ($p < 0.05$) negative irrespective of N level.

Table 1. Mean values of hybrids (H) and inbred lines (I) at high nitrogen (HN) and low nitrogen (LN) availability and percentage change relative to HN for traits related to phenology and light capture.

		Experiments with predominantly significant N effect (Exp. 1 to 5)			Experiments with predominantly no significant N effect					
					Additional heat stress at CP† (Exp. 6)			Additional drought stress (Exp. 7)		
Trait‡	Genotype	HN	LN	% Change	HN	LN	% Change	HN	LN	% Change
TT to anthesis (°C d^{-1})	H	897 b§	919 b	2 NS¶	930 b	936 b	0.6 NS	1032 b	1040 a	1 NS
	I	970 a	988 a	2 NS	1037 a	1033 a	−0.4 NS	1144 a	1135 b	−1 NS
TT to silking (°C d^{-1})	H	915 b	957 b	5***	984 b	984 b	0.1 NS	1112 b	1110 b	−0.2 NS
	I	997 a	1028 a	3*	1089 a	1084 a	−0.5 NS	1204 a	1205 a	0.1 NS
ASI (day)	H	1.07 b	2.59 b	1.5***#	3.97 a	3.71 a	−0.3 NS	6.18 a	6.05 a	−0.1 NS
	I	2.01 a	2.82 a	0.8 NS	3.76 a	3.89 a	−0.1 NS	5.55 a	5.11 a	−0.4 NS
LAI_{MAX}	H	4.63 a	3.31 a	−29***	4.95 a	5.01 a	1 NS	4.38 a	4.08 a	−7*
	I	3.26 b	2.63 b	−19***	3.73 b	3.40 b	−10 NS	3.51 b	3.21 b	−9 NS
LAI_{PM}	H	3.36 a	1.20 a	−64***	2.94 a	2.57 a	−14 NS	1.49 a	1.18 a	−21 NS
	I	1.49 b	1.01 b	−32***	1.68 b	1.61 b	−4 NS	1.01 b	1.08 a	7 NS
$fIPAR_{MAX}$	H	0.89 a	0.76 a	−15***	0.95 a	0.93 a	−2*	0.87 a	0.86 a	−1 NS
	I	0.72 b	0.60 b	−17***	0.84 b	0.79 b	−6**	0.70 b	0.68 b	−3 NS
$fIPAR_{PM}$	H	0.71 a	0.36 a	−49***	0.63 a	0.59 a	−7 NS	0.26 a	0.26 a	0 NS
	I	0.36 b	0.27 b	−25***	0.42 b	0.39 b	−8 NS	0.17 b	0.20 b	−18 NS
$IPARi_{CP}$††	H	9.90 a	8.40 a	−15***	10.40 a	10.10 a	−3**	9.31 a	9.07 a	−3 NS
	I	7.70 b	6.45 b	−16***	9.32 b	8.86 b	−5*	7.98 b	7.36 b	−8*
$IPARi_{GF}$††	H	391 a	239 a	−39***	490 a	469 a	−5**	331	317	−4 NS
	I	215 b	160 b	−26***	395 b	372 b	−6 NS	226	212	−6 NS
$IPARi_{PM}$††	H	814 a	506 a	−38***	860 a	819 a	−4**	571 a	554 a	−3 NS
	I	593 b	466 b	−21***	647 b	624 b	−4 NS	445 b	417 b	−6 NS

*Significant at the 0.05 probability level.

**Significant at the 0.01 probability level.

***Significant at the 0.001 probability level.

†CP, critical period.

‡TT, thermal time; ASI, anthesis-silking interval; LAI_{MAX}, maximum leaf area index; LAI_{PM}, leaf area index at physiological maturity; $fIPAR_{MAX}$, maximum fraction of incident photosynthetically active radiation intercepted by canopy ; $fIPAR_{PM}$, fraction of incident photosynthetically active radiation intercepted by canopy at physiological maturity; $IPARi_{CP}$, intercepted incident photosynthetically active radiation during the critical period; $IPARi_{GF}$, intercepted incident photosynthetically active radiation during the grain-filling period; $IPARi_{PM}$, intercepted incident photosynthetically active radiation during the whole cycle.

§Within each column and trait, means followed by the same letter are not significantly different at $p < 0.05$.

¶NS = not significant.

#Single trait represented in absolute value.

††Only for Exp. 1 to 3, 6, and 7.

When overall abiotic stress was intensified due to high temperature, heterosis for RUE was similar or higher under LN than HN level, opposite to the response pattern mentioned for experiments were no additional stress occurred other than LN (Table 2). Similar response was observed for biomass production and PGR_{CP}. With the occurrence of an additional drought stress no significant heterosis for $Biomass_{V14}$, EGR_{CP}, RUE_{GF}, and RUE_{PM} was found. In addition, the differences in heterosis mentioned for biomass accumulation between N treatments did not hold (Table 2). That is, hybrid advantage for $Biomass_{V14}$ was not significant at either level of N supply, and biomass at R_2 and PGR_{CP} had higher mean values of heterosis at LN than at HN availability (26 and 19%, respectively). Heterosis for traits such as RUE and EGR_{CP} PGR_{CP}^{-1} ratio was, in general, negative and not significant.

Grain Yield and Its Numerical Components

Plant grain yield, its numerical components (i.e., KNP and KW), and both plant and ear reproductive capacities were higher in hybrids than in inbreds regardless of N availability. Plant grain yield significantly decreased with LN, but hybrids were more responsive than inbred lines (Table 3). Increases in PGY under HN were greater for hybrids (65%) than in inbred lines (30%). High N also increased mean KNP and KW of hybrids by 41 and 18%, respectively, whereas KNP of inbred lines increased to a lesser extent (25%) and no significant KW response to HN was observed for this group.

When N deficiency was the predominant stress condition, described differences between groups in the relative response to LN affected heterosis values accordingly. Mean heterosis for PGY, KNP, and KW was significant ($p < 0.001$) at both N levels, but higher values ($p < 0.01$) were registered at HN (137, 76, and 35%, respectively) than at LN conditions (87, 56, and 22%, respectively).

The response of plant and ear reproductive capacities to N treatments was, in general, not significant and opposite to that mentioned for PGY and KNP. Heterosis for these traits (Table 2), however, was observed and was highly

Table 2. Mean percentage and range of heterosis based on midparent values at high nitrogen (HN) and low nitrogen (LN) availability for traits related to phenology, light capture, and biomass production and its partitioning and grain yield determination.

	Experiments with predominantly significant N effect (Exp. 1 to 5)				Experiments with predominantly no significant N effect							
					Additional heat stress at CP† (Exp. 6)				Additional drought stress (Exp. 7)			
	HN		LN		HN		LN		HN		LN	
Trait‡	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
TT to anthesis (°C d^{-1})	–7***	–14 to 13	–6***	–13 to 14	–10***	–12 to –7	–9***	–10 to –6	–10***	–11 to –7	–8***	–11 to –8
TT to silking (°C d^{-1})	–7***	–16 to 17	–6***	–13 to 13	–9***	–11 to –4	–8***	–10 to –3	–5**	–8 to –1	–6**	–9 to –3
ASI (day)¶	–0.6 NS§	2.6 to 1.6	0.1 NS	–2.3 to 1.5	0.4 NS	–1 to 2	0.2 NS	–2 to 2	1.2 NS	0.1 to 3	2.0 NS	0.4 to 5
LAI_{MAX}	51***	29 to 82	32***	5 to 66	45***	18 to 60	56***	28 to 87	37***	5 to 61	38***	2 to 61
LAI_{PM}	134***	50 to 428	23***	–84 to 175	72**	42 to 151	66*	25 to 194	33*	–47 to 95	–2 NS	–83 to 85
$fIPAR_{MAX}$	28***	13 to 43	31***	8 to 52	15***	10 to 18	17***	12 to 20	22*	12 to 35	31***	16 to 40
$fIPAR_{PM}$	112***	22 to 441	38 NS	–75 to 160	40**	30 to 54	42*	12 to 102	42 NS	6 to 88	26 NS	–22 to 102
$IPARi_{CP}$#	32***	15 to 52	31***	16 to 43	14 NS	8 to 16	12 NS	9 to 19	16***	7 to 29	26***	12 to 35
$IPARi_{GF}$#	86***	45 to 150	49***	16 to 98	22***	20 to 26	23***	18 to 31	45***	34 to 67	51***	42 to 59
$IPARi_{PM}$#	49***	26 to 70	35***	24 to 53	33***	24 to 38	29***	21 to 38	28***	16 to 37	34***	21 to 46
RUE_{V14}#	30**	–6 to 72	9**	–22 to 64	3**	–19 to 14	28*	7 to 47	3 NS	–19 to 14	28**	7 to 47
RUE_{CP}#	20 NS	–12 to 57	–5 NS	–23 to 20	37***	20 to 56	34***	7 to 62	–10*	–47 to 18	–21***	–32 to –1
RUE_{GF}#	34**	4 to 78	16 NS	–29 to 86	52***	32 to 86	61***	27 to 77	16 NS	–7 to 48	–12 NS	–25 to –1
RUE_{PM}#	24**	7 to 34	4 NS	–11 to 31	28***	13 to 50	40***	31 to 48	2 NS	–11 to 15	–8 NS	–21 to 5
$Biomass_{V14}$ (g $plant^{-1}$)	131***	38 to 252	96**	21 to 208	34**	–1 to 56	45***	21 to 81	2 NS	–32 to 20	7 NS	12 to 37
$Biomass_{R2}$ (g $plant^{-1}$)	59***	24 to 88	38***	–2 to 92	44***	18 to 63	46***	31 to 79	19*	–11 to 42	26**	12 to 47
$Biomass_{PM}$ (g $plant^{-1}$)	80***	49 to 127	45***	23 to 103	71***	45 to 106	82***	58 to 105	34**	9 to 52	26*	18 to 37
PGR_{CP} (g $plant^{-1}$ day^{-1})	65***	19 to 130	38**	–20 to 154	49***	30 to 85	54***	28 to 95	35***	10 to 83	44***	17 to 65
EGR_{CP} (g d^{-1})	48***	–16 to 113	20 NS	–38 to 76	37**	12 to 67	30*	–2 to 53	24 NS	–19 to 76	30 NS	4 to 44
EGR_{CP} PGR_{CP}^{-1}	–9*	–35 to 47	–11*	–41 to 32	–8 NS	–19 to 6	–13*	–50 to 9	–9 NS	–25 to 12	–9 NS	–38 to 19
KNP ($plant^{-1}$)	76***	21 to 150	56***	–5 to 143	108***	56 to 149	63***	42 to 87	69 NS	–18 to 188	51*	19 to 88
KNP PGR_{CP}^{-1}	7 NS	–38 to 62	21*	–29 to 83	13 NS	–2 to 28	43**	–1 to 108	27 NS	–25 to 96	10 NS	–30 to 40
KNE_1 EGR_{CP}^{-1}	23**	–22 to 67	34***	–25 to 107	54***	20 to 84	87***	10 to 137	60 NS	–8 to 148	28 NS	–12 to 50
KW (g $kernel^{-1}$)	35***	20 to 48	22***	10 to 37	25***	16 to 29	35***	29 to 39	8 NS	–11 to 20	3 NS	–6 to 10
HI	31***	1 to 67	28***	–9 to 89	50***	26 to 72	33***	20 to 41	39 NS	–6 to 74	22*	–24 to 73
PGY (g $plant^{-1}$)	137***	72 to 254	87***	23 to 208	167***	88 to 219	119***	86 to 154	59*	12 to 92	70*	–17 to 138

*Significant at the 0.05 probability level.

**Significant at the 0.01 probability level.

***Significant at the 0.001 probability level.

†CP, critical period.

‡TT, thermal time; ASI, anthesis-silking interval; LAI_{MAX}, maximum leaf area index; LAI_{PM}, leaf area index at physiological maturity; $fIPAR_{MAX}$, maximum fraction of incident photosynthetically active radiation intercepted by canopy ; $fIPAR_{PM}$, fraction of incident photosynthetically active radiation intercepted by canopy at physiological maturity; $IPARi_{CP}$, intercepted incident photosynthetically active radiation during the critical period; $IPARi_{GF}$, intercepted incident photosynthetically active radiation during the grain-filling period; $IPARi_{PM}$, intercepted incident photosynthetically active radiation during the whole cycle; RUE_{V14}, radiation use efficiency up to V_{14}; RUE_{CP}, radiation use efficiency during the critical period; RUE_{GF}, radiation use efficiency during the grain-filling period; RUE_{PM}, radiation use efficiency at physiological maturity; $Biomass_{V14}$, aboveground biomass at V_{14}; $Biomass_{R2}$, aboveground biomass at R_2; $Biomass_{PM}$, aboveground biomass at physiological maturity; PGR_{CP}, plant growth rate during the critical period; EGR_{CP}, apical ear growth rate during the critical period; KNP, kernel number per plant; KNE_1, kernel number per apical ear; KW, kernel weight; HI, harvest index; PGY, plant grain yield.

§NS = not significant.

¶Absolute value of heterosis.

#Only for Exp. 1 to 3, 6, and 7.

significant ($p < 0.01$) at both N levels for ear reproductive capacity (23% at HN and 34% at LN) and only moderately significant ($p < 0.05$) at LN (21%) for plant reproductive capacity. The occurrence of an additional drought stress masked the expression of heterosis for reproductive capacities, regardless of N treatment (Table 2).

Genotype by Trait Association for Heterosis in Response to Nitrogen

When it focused on the response of genotypes and attributes at Exp. 1 to 5 (i.e., under predominant N effect), heterosis for PGY was strongly and positively correlated ($r > 0.70$; $p < 0.001$) with heterosis for KNP, $Biomass_{PM}$, HI, and RUE_{GF} irrespective of N condition (Table 4). Similarly, heterosis for traits related to light capture around flowering (i.e., $IPARi_{CP}$, LAI_{MAX}, and $fIPAR_{MAX}$), $Biomass_{R2}$, and both reproductive capacities were positively associated ($0.35 > r \leq 0.70$; $p < 0.05$) with heterosis for PGY, and many of these correlations were stronger under LN than at HN (Table 4). However, some traits modified their response depending on N level; for example, heterosis for PGY was associated ($p < 0.05$) with heterosis for EGR_{CP} ($r = 0.57$), RUE_{CP} ($r = 0.52$), KW ($r = 0.44$), and ASI ($r = -0.37$) only at LN.

Table 3. Mean values of hybrids (H) and inbred lines (I) at high nitrogen (HN) and low nitrogen (LN) availability and percentage change relative to HN for traits related to biomass production and its partitioning and grain yield determination.

		Experiments with predominantly significant N effect (Exp. 1 to 5)			Experiments with predominantly no significant N effect					
					Additional heat stress at CP† (Exp. 6)			Additional drought stress (Exp. 7)		
Trait‡	Genotype	HN	LN	% Change	HN	LN	% Change	HN	LN	% Change
RUE_{V14}§	H	3.59 a¶	3.14 a	–12**	3.35 a	3.18 a	–5 NS#	2.96 b	2.68 b	–9 NS
	I	3.07 b	2.83 b	–7 NS	2.77 b	2.70 b	–2 NS	3.65 a	3.27 a	–10*
RUE_{CP}§	H	3.04 a	2.41 a	–21***	2.86 a	2.88 a	0.7 NS	2.18 b	2.60 b	19**
	I	2.52 b	2.54 a	1 NS	2.23 b	2.05 b	–9 NS	2.63 a	3.13 a	19 NS
RUE_{GF}§	H	2.34 a	1.84 a	–21***	3.13 a	3.30 a	5 NS	2.22 a	1.98 b	–11 NS
	I	1.67 b	1.56 b	–7 NS	2.06 b	2.05 b	–0.5 NS	1.81 b	2.16 a	19 NS
RUE_{PM}§	H	2.83 a	2.31 a	–18***	2.34 a	2.45 a	4 NS	1.97 a	1.87 a	–5 NS
	I	2.26 b	2.22 a	–2 NS	1.83 b	1.74 b	–5 NS	2.00 a	1.99 a	–1 NS
$Biomass_{V14}$ (g $plant^{-1}$)	H	81.8 a	58.1 a	–33***	67.4 a	66.7 a	–1 NS	59.3 a	52.8 a	–11*
	I	40.2 b	32.9 b	–20*	54.3 b	47.7 b	–12 NS	64.3 a	53.9 a	–16*
$Biomass_{R2}$ (g $plant^{-1}$)	H	183 a	128 a	–30***	200 a	194 a	–3 NS	159 a	152 a	–5 NS
	I	120 b	97.5 b	–19***	150 b	131 b	–13*	139 b	125 b	–10 NS
$Biomass_{PM}$ (g $plant^{-1}$)	H	290 a	186 a	–36***	288 a	287 a	–0.1 NS	161 a	151 a	–6 NS
	I	163 b	130 b	–20***	171 b	156 b	–9 NS	121 b	119 b	–2 NS
PGR_{CP} (g $plant^{-1}$ day^{-1})	H	4.00 a	2.67 a	–33***	4.88 a	4.15 a	–15 NS	3.45 a	3.29 a	–5 NS
	I	2.49 b	2.10 b	–16***	2.85 b	2.69 b	–6 NS	2.45 b	2.25 b	–8 NS
EGR_{CP} (g d^{-1})	H	1.21 a	0.81 a	–18***	2.46 a	2.36 a	–4 NS	1.41 a	1.61 a	14 NS
	I	0.89 b	0.73 b	–18***	1.95 b	1.86 b	–5 NS	1.09 a	1.18 b	8 NS
EGR_{CP} PGR_{CP}^{-1}	H	0.31 a	0.31 a	0 NS	0.56 a	0.57 a	2 NS	0.39 a	0.47 a	21*
	I	0.36 b	0.35 b	–3 NS	0.65 b	0.69 b	6 NS	0.42 a	0.52 a	24 NS
KNP ($plant^{-1}$)	H	460 a	325 a	–29***	417 a	435 a	4 NS	2231 a	249 a	11 NS
	I	280 b	224 b	–20***	268 b	226 b	–16 NS	1221 b	158 b	29 NS
KNP PGR_{CP}^{-1}	H	127 a	119 a	–6*	98.4 a	107 a	9*	60.3 a	74.6 a	24*
	I	117 b	106 b	–9 NS	89.0 a	86.9 b	–2 NS	43.1 a	67.6 a	57*
KNE_1 EGR_{CP}^{-1}	H	396 a	425 a	7**	200 a	188 a	–6 NS	154 a	159 a	3 NS
	I	312 b	316 b	1 NS	126 b	122 b	–3 NS	83.7 b	115 b	37 NS
KW (g $kernel^{-1}$)	H	280 a	238 a	–15***	259 a	253 a	–3 NS	149 a	153 a	2 NS
	I	201 b	191 b	–5 NS	191 b	199 b	5 NS	141 a	137 b	3 NS
HI	H	0.44 a	0.41 a	–7 NS	0.37 a	0.39 a	3 NS	0.21 a	0.26 a	25*
	I	0.34 b	0.32 b	–6 NS	0.29 b	0.28 b	–3 NS	0.14 b	0.18 b	29 NS
PGY (g $plant^{-1}$)	H	129 a	77.8 a	–39***	108 a	111 a	2 NS	34.0 a	38.8 a	14 NS
	I	56.4 b	43.3 b	–23***	51.8 b	44.6 b	–14 NS	17.7 b	22.7 b	28 NS

*Significant at the 0.05 probability level.

**Significant at the 0.01 probability level.

***Significant at the 0.001 probability level.

†CP, critical period.

‡RUE_{V14}, radiation use efficiency up to V_{14}; RUE_{CP}, radiation use efficiency during the critical period; RUE_{GF}, radiation use efficiency during the grain-filling period; RUE_{PM}, radiation use efficiency at physiological maturity; $Biomass_{V14}$, aboveground biomass at V_{14}; $Biomass_{R2}$, aboveground biomass at R_2; $Biomass_{PM}$, aboveground biomass at physiological maturity; PGR_{CP}, plant growth rate during the critical period; EGR_{CP}, apical ear growth rate during the critical period; KNP, kernel number per plant; KNE_1, kernel number per apical ear; KW, kernel weight; HI, harvest index; PGY, plant grain yield.

§Only for Exp. 1 to 3, 6, and 7.

¶Within each column and trait, means followed by the same letter are not significantly different at $p < 0.05$.

#NS = not significant.

Principal component analysis explored the genotype by trait interaction in each N condition. The first two components (i.e., PC1 and PC2) accounted for 63 and 71% of the variation for LN (Fig. 2A) and HN (Fig. 2B), respectively. The strength of correlation (i.e., angle between trait vectors) varied substantially depending on which traits were compared. The most significant correlations were observed for traits that share a common physiological basis. The acute angles formed by the attribute vectors of HI, KNP, $Biomass_{PM}$, $RUE_{GF,}$ LAI_{MAX}, and $Biomass_{R2}$ with PGY in both biplots indicated that the mean heterosis for these traits were strongly and positively associated with heterosis for PGY across all genotypes (Fig. 2), as mentioned in the previous paragraph.

Under LN (Fig. 2A), heterosis for traits related to plant growth during the critical period (i.e., PGR_{CP} and EGR_{CP}) and RUE_{PM} were also strongly and positively associated with heterosis for PGY. Heterosis for PGY was not associated

Table 4. Phenotypic correlation (*r*) between percentage of heterosis for plant grain yield and percentage of heterosis for physiological traits cropped at two N levels (high nitrogen [HN] and low nitrogen [LN]) in Exp. 1 to 5.

Trait†	HN	LN
TT to anthesis (°C d^{-1})	−0.06 NS‡	−0.11 NS
TT to silking (°C d^{-1})	−0.05 NS	−0.13 NS
ASI (day)§	−0.23 NS	−0.37*
LAI_{MAX}	0.50**	0.69***
LAI_{PM}	0.04 NS	0.03 NS
$fIPAR_{MAX}$	0.47**	0.51***
$fIPAR_{PM}$	0.04 NS	−0.05 NS
$IPARi_{CP}$¶	0.62**	0.59**
$IPARi_{GF}$¶	0.27 NS	0.04 NS
$IPARi_{PM}$¶	0.30 NS	0.05 NS
RUE_{V14}¶	0.47***	0.37**
RUE_{CP}¶	0.35 NS	0.52*
RUE_{GF}¶	0.77***	0.92***
RUE_{PM}¶	0.01 NS	0.38 NS
$Biomass_{V14}$ (g $plant^{-1}$)	0.43*	0.35 NS
$Biomass_{R2}$ (g $plant^{-1}$)	0.46**	0.58***
$Biomass_{PM}$ (g $plant^{-1}$)	0.73***	0.75***
PGR_{CP} (g $plant^{-1}$ day^{-1})	0.12 NS	0.29 NS
EGR_{CP} (g d^{-1})	0.32 NS	0.57***
EGR_{CP} PGR_{CP}^{-1}	0.32 NS	0.28 NS
KNP ($plant^{-1}$)	0.93***	0.96***
KNP PGR_{CP}^{-1}	0.64***	0.57***
KNE_1 EGR_{CP}^{-1}	0.38*	0.37*
KW (g $kernel^{-1}$)	0.29 NS	0.44**
HI	0.80***	0.85***

*Significant at the 0.05 probability level.

**Significant at the 0.01 probability level.

***Significant at the 0.001 probability level.

†TT, thermal time; ASI, anthesis-silking interval; LAI_{MAX}, maximum leaf area index; LAI_{PM}, leaf area index at physiological maturity; $fIPAR_{MAX}$, maximum fraction of incident photosynthetically active radiation intercepted by canopy; $fIPAR_{PM}$, fraction of incident photosynthetically active radiation intercepted by canopy at physiological maturity; $IPARi_{CP}$, intercepted incident photosynthetically active radiation during the critical period; $IPARi_{GF}$, intercepted incident photosynthetically active radiation during the grain-filling period; $IPARi_{PM}$, intercepted incident photosynthetically active radiation during the whole cycle; RUE_{V14}, radiation use efficiency up to V_{14}; RUE_{CP}, radiation use efficiency during the critical period; RUE_{GF}, radiation use efficiency during the grain-filling period; RUE_{PM}, radiation use efficiency at physiological maturity; $Biomass_{V14}$, aboveground biomass at V_{14}; $Biomass_{R2}$, aboveground biomass at R_2; $Biomass_{PM}$, aboveground biomass at physiological maturity; PGR_{CP}, plant growth rate during the critical period; EGR_{CP}, apical ear growth rate during the critical period; KNP, kernel number per plant; KNE_1, kernel number per apical ear; KW, kernel weight; HI, harvest index.

‡NS = not significant.

§Absolute value.

¶Only Exp. 1 to 3.

with ASI and reproductive capacities. Similarly, no association between heterosis for KNP and KW was found. The different arrangement of genotypes and attributes allowed differentiating hybrids with contrasting response pattern. Genotype B100 × LP561 showed a positive perpendicular projection on the PGY vector and high relative heterosis for both KNP and KW (Fig. 2A). In contrast, genotype ZN6 × LP611 had a similar projection on the PGY vector with high relative heterosis for KNP but negative for KW. Hybrid B100 × LP2 showed an opposite response to ZN6 × LP611 (i.e., high heterosis for KW and negative for KNP).

Under HN (Fig. 2B), heterosis for traits related to light capture (i.e., $IPARi_{CP}$, $IPARi_{GF}$, and $IPARi_{PM}$) was also strongly associated with heterosis for PGY. Slightly weaker associations were recorded between heterosis for PGY and heterosis for PGR_{CP}, RUE_{CP}, RUE_{PM}, KW, and ear and plant reproductive capacities. In contrast, heterosis for ASI was negatively associated and no association was found with heterosis for EGR_{CP} and canopy size at maturity (i.e., LAI_{PM} and $fIPAR_{PM}$). Nitrogen levels modified the ranking order of hybrids regarding heterosis for PGY. At HN conditions ZN6 × LP611 and B100 × LP2 showed the highest relative values of heterosis for grain yield and ecophysiological related traits. Moreover, genotype ZN6 × LP611 presented the highest values of heterosis for RUE_{CP} and reproductive capacities (Fig. 2B). Genotypes LP561 × LP662 and ZN6 × LP561 (including reciprocals) had a similar response pattern showing the lowest relative values of heterosis for PGY and ecophysiological related traits (i.e., negative perpendicular projection on PC1 axis), regardless of N availability, that is, they consistently showed the lowest values of hybrid vigor across traits (Fig. 3). Genotypes that had B100 as parental line (either as female or male parent) always had positive scores on PC1 and greater heterosis for traits highly correlated with heterosis for PGY (Fig. 2 and 3). Moreover, genotype B100 × LP2 had the highest overall heterosis under both LN and HN availability (Fig. 3) and was always among the 10% highest yielding hybrids (data not shown). In contrast, Hybrid LP561 × LP662 showed the opposite response (Fig. 3).

DISCUSSION

The results of the present study showed that the expression of heterosis for PGY and ecophysiological related traits varied across years (Tollenaar et al., 2004) and resource availability, that is, single (N) or simultaneous occurrence of abiotic stress (e.g., water × N, heat × N interaction) can differentially affect heterosis for PGY and ecophysiological related traits in maize. This suggests that changes in heterosis with stress highly depend on the nature of the environmental stress. In general, heterosis decreased under abiotic restrictions that had a predominant effect of reducing tissue expansion first (summarized in LAI_{MAX}) and photosynthetic capacity next (summarized in RUEs), as for N or water deficits that took place during active vegetative growth before silking. This response of heterosis can also be explained by the reduced effect of these stresses on inbreds as compared to hybrids (D'Andrea et al., 2009), attributable to the intrinsic reduced demand of resources from tissues already affected in their expansion by the negative effects of inbreeding. Nevertheless, the particular combination of timing × intensity × development of different stress types, or the co-occurrence of stresses that have an opposite effect on the physiological determinants of grain yield (e.g., heat stress has a larger effect on RUE than on leaf expansion; Cicchino et al., 2010), affected the response of each genotype group differentially, with the concomitant

variation in heterosis expression. Therefore, a first approach in the interpretation of heterosis results will be considering the effects of N as a single stress factor followed by a comparative analysis of the co-occurrence of different abiotic stresses.

Heterosis and Nitrogen Supply

In contrast to reports in literature that suggest an increase in heterosis with high plant density or water stress during the critical period bracketing silking (Echarte and Tollenaar, 2006; Liu and Tollenaar, 2009b), LN stress did not enhance the expression of heterosis in hybrids. The highest observed level of mean heterosis corresponded to PGY (137%) followed closely by LAI_{PM} (134%) and $Biomass_{V14}$ (131%), all at the HN condition. These results support the general consensus on the larger negative response to reduced N supply of hybrids as compared to inbreds for grain yield (Betran et al., 2003; Zaidi et al., 2003) and ecophysiological related traits (D'Andrea et al., 2009), because of the already mentioned low resource requirement of inbreds due to their intrinsic reduced maximum growth. This differential effect of N deficiency on the F_1 progeny and the parental inbreds should reduce the expression of heterosis for crops growing under this constraint, as computed from our results. In the current research, differences between hybrids and inbreds were greater under HN than LN, in particular for traits related to canopy size (i.e., in green leaf area index [LAI]), light capture, and biomass accumulation, in agreement with our previous reports on this topic (D'Andrea et al., 2009). Meristematic regions develop more rapidly in hybrids than in their parents and in direct proportion to the amount of N supplied (Burkholder and McVeigh, 1940). Considering the interrelationships between source and sink activity, when the supply of N is poor, relative growth rate is more frequently restricted by rate of cell production (i.e., sink activity) than by carbohydrate supply (i.e., source activity) (Sugiyama and Gotoh, 2009); therefore, an accumulation of starch representing a shortage of sink activity relative to source function may occur with a negative feedback effect within the leaf on leaf photosynthesis (Gifford and Evans, 1981; Prioul, 1996), thus reducing the effect of LN on heterosis for leaf photosynthesis and ultimately RUE. We detected that mean reductions in PGY in response to LN availability (39% for hybrids and 23% for inbreds) may be ascribed to similar reductions in $Biomass_{PM}$ (36 and 20%, respectively) as no significant decrease in HI was found. Therefore, the dominant effect of N supply was on biomass production (Ding et al., 2005; D'Andrea et al., 2009; Massignam et al., 2010) rather than on its partitioning to the

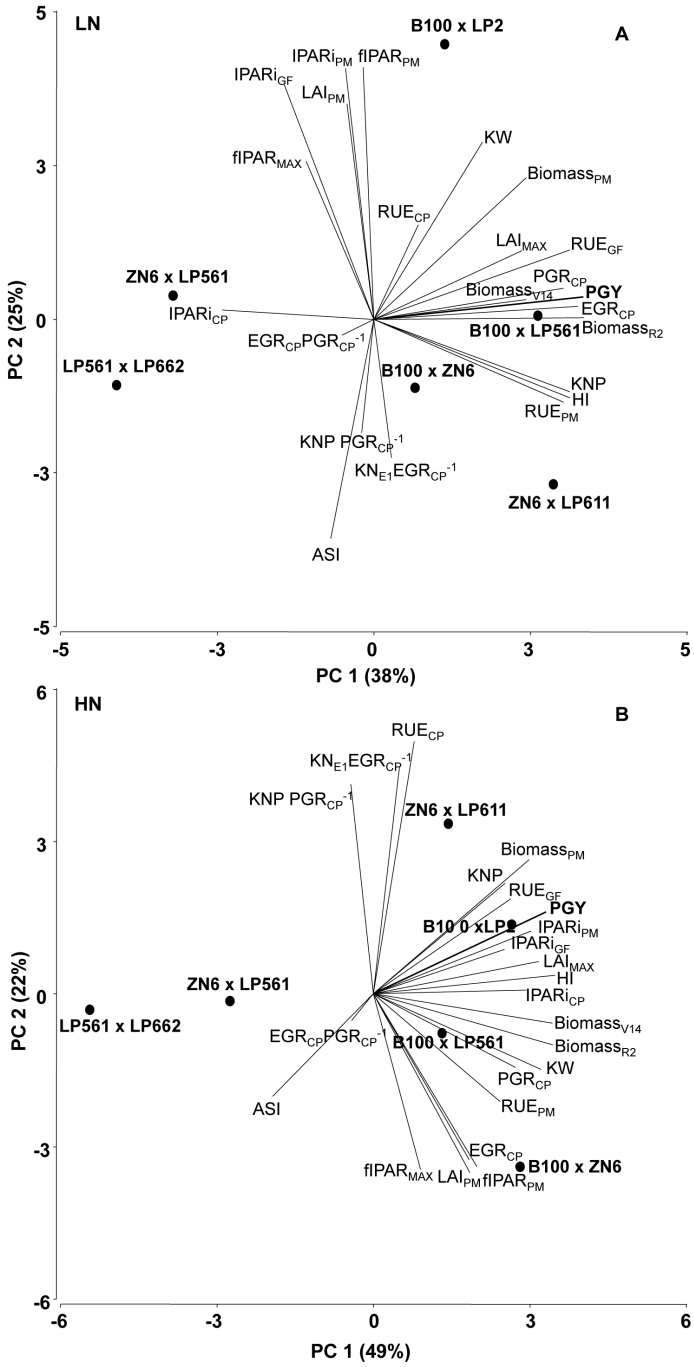

Figure 2. Biplots for the first two principal components from the analysis of 12 hybrids evaluated for heterosis for 23 traits at low nitrogen (LN) and high nitrogen (HN) availability for Exp. 1 to 5. Traits are represented by vectors and genotypes by points. PC1 and PC2, first two principal components; ASI, anthesis-silking interval; $Biomass_{PM}$, aboveground biomass at physiological maturity; $Biomass_{R2}$, aboveground biomass at R_2; $Biomass_{V14}$, aboveground biomass at V_{14}; EGR_{CP}, apical ear growth rate during the critical period; $fIPAR_{MAX}$, maximum fraction of incident photosynthetically active radiation intercepted by canopy; $fIPAR_{PM}$, fraction of incident photosynthetically active radiation intercepted by canopy at physiological maturity ; HI, harvest index; $IPARi_{CP}$, intercepted incident photosynthetically active radiation during the critical period; $IPARi_{GF}$, intercepted incident photosynthetically active radiation during the grain-filling period; $IPARi_{PM}$, intercepted incident photosynthetically active radiation during the whole cycle; KNE_1, kernel number per apical ear; KNP, kernel number per plant; KW, kernel weight; LAI_{MAX}, maximum leaf area index; LAI_{PM}, leaf area index at physiological maturity; PGR_{CP}, plant growth rate during the critical period; PGY, plant grain yield; RUE_{CP}, radiation use efficiency during critical period; RUE_{GF}, radiation use efficiency during the grain-filling period; RUE_{PM}, radiation use efficiency at physiological maturity.

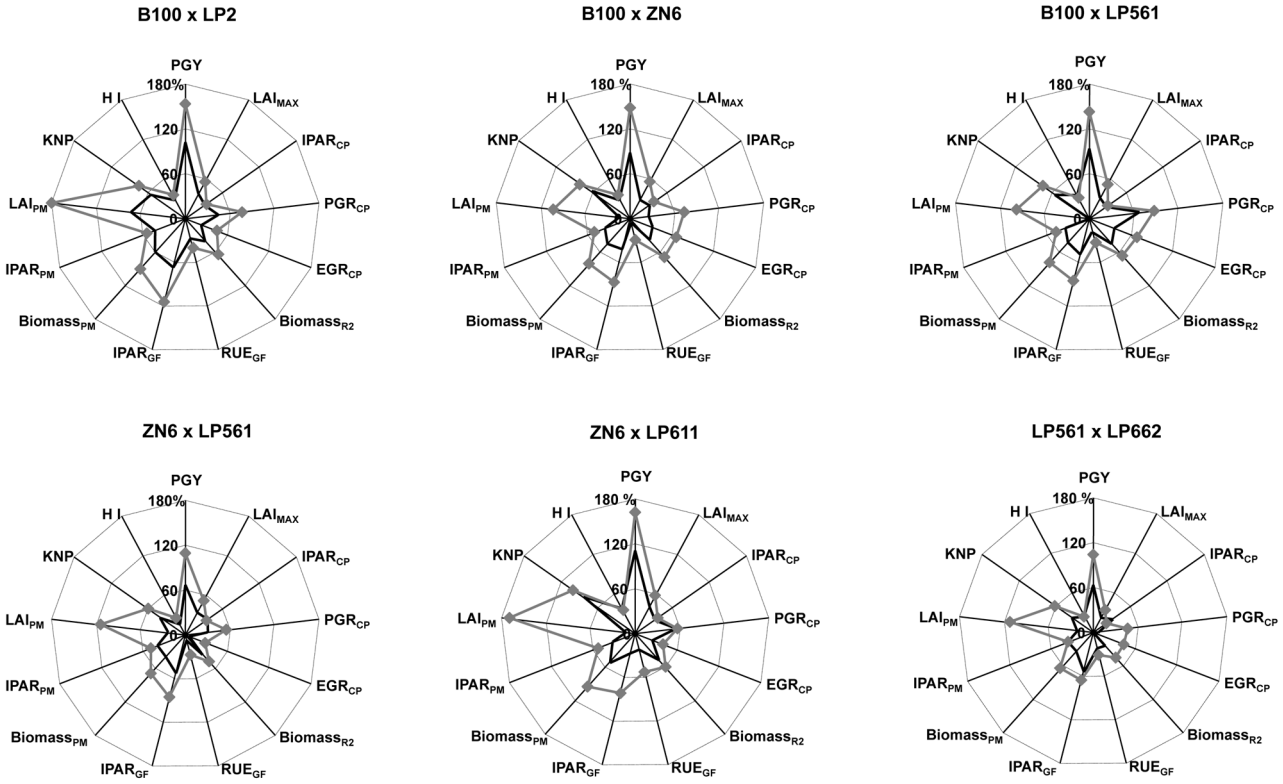

Figure 3. Mean midparent heterosis (MPH) for multiple traits and F_1 hybrids (including reciprocals) at low nitrogen (LN) and high nitrogen (HN). The concentric circles represent mean % MPH. Each spoke is a trait and contains two values of MPH percentage: Low nitrogen (solid line) and HN (gray marker line). PGY, plant grain yield; LAI_{MAX}, maximum leaf area index; $IPARi_{CP}$, intercepted incident photosynthetically active radiation during the critical period; PGR_{CP}, plant growth rate during the critical period; EGR_{CP}, apical ear growth rate during the critical period; $Biomass_{R2}$, aboveground biomass at R_2; RUE_{GF}, radiation use efficiency during the grain-filling period; $IPARi_{GF}$, intercepted incident photosynthetically active radiation during the grain-filling period; $Biomass_{PM}$, aboveground biomass at physiological maturity; $IPARi_{PM}$, intercepted incident photosynthetically active radiation during the whole cycle; LAI_{PM}, leaf area index at physiological maturity; KNP, kernel number per plant; HI, harvest index.

ear. This result is consistent with studies regarding changes in plant traits after selection for grain yield in both LN and HN environments, which increased total biomass but did not change HI (Laffite and Edmeades, 1994).

The effect of N supply on heterosis for IPARi and biomass accumulation was more evident during the grain-filling period than for previous growth stages. Although the greatest difference in biomass accumulation between hybrids and their parental inbred lines was seen at V_{14} under both N levels, its contribution to total aboveground biomass at maturity for hybrids was small (less than 35%) and similar to that found in maize grown under optimum conditions (Tollenaar et al., 2004). With the advance in crop cycle the differences in heterosis for biomass accumulation between N treatments became more pronounced. The increase in heterosis for RUE during the grain-filling period at HN is in agreement with previous findings of an increase in heterosis for carbon exchange rate from 2 wk postsilking onward (Ahmadzadeh et al., 2004). The greater discrepancy in heterosis for biomass accumulation between LN and HN at physiological maturity than at earlier stages and the increase in heterosis for RUE during grain filling may suggest that the major effects of N shortage on grain yield in hybrids in this study occurred mostly during the postsilking period and could be attributed to a markedly reduced reproductive sink strength caused by the large decrease in seed numbers (Sadras et al., 2000; D'Andrea et al., 2009). The greater PGR_{CP} of hybrids relative to inbreds under both levels of N availability (on average 50%) can be explained by a greater LAI_{MAX} (40%) and $IPARi_{CP,}$ (30%) as no significant heterosis for RUE_{CP} was observed. This agrees with studies indicating that the effects of inbreeding depression are more related to leaf expansion and light interception than to radiation use efficiency (D'Andrea et al., 2006). Heterosis for PGR_{CP} translated into significant heterosis for plant reproductive capacity under LN only, suggesting that N deficiency in inbred lines may limit kernel set not only indirectly (i.e., through plant growth rate) but through direct effects on N metabolism in developing grains (Seebauer et al., 2004; D'Andrea et al., 2008). Despite that heterosis for EGR_{CP} under LN was not statistically significant, hybrids showed higher ear reproductive capacity than inbreds. This response can be attributed to differences between the two groups in the pattern of biomass allocation among tissues within the ear (i.e., reproductive vs. vegetative) or in the developmental pattern among florets, which were not addressed in the current study and will need attention in future research.

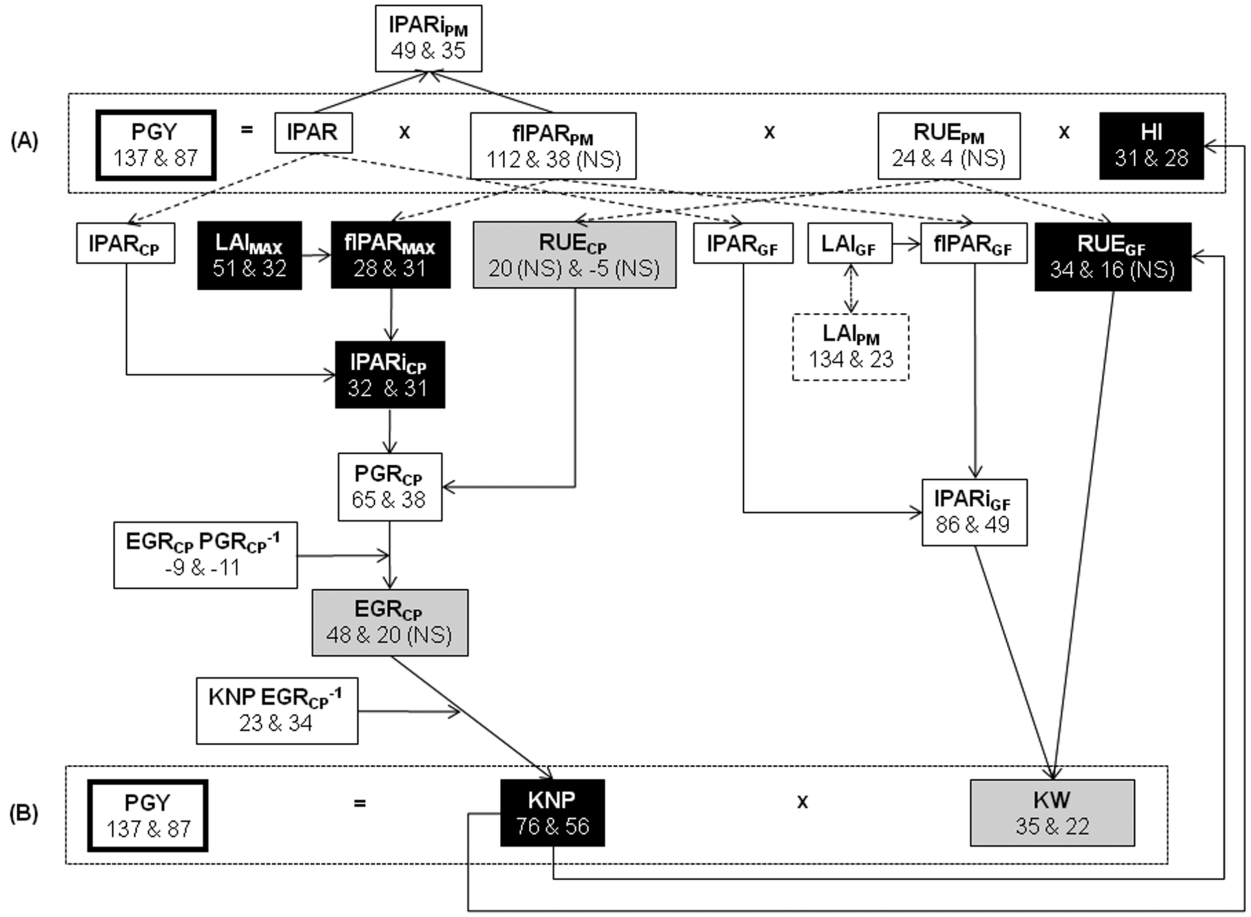

Figure 4. Flow diagram linking main physiological determinants of grain yield (A) and grain yield components (B). Dashed arrows link variables of the same group (i.e., light interception efficiency or radiation use efficiency). Solid arrows indicate direct effects. Numbers for each variable represent percent heterosis at high and low N level (X_1 and X_2, respectively), when N was the single limiting factor (Table 2). Heterosis was not computed for leaf area index (LAI) and fraction of daily amount of incident photosynthetically active radiation intercepted by canopy (fIPAR) during grain filling (LAI_{GF} and $fIPAR_{GF}$, respectively) and data for LAI at physiological maturity (LAI_{PM}) are given as indirect reference of the former. Variables for which computed heterosis was significantly (Table 4) correlated with plant grain yield (PGY) heterosis at all N levels are indicated with black boxes. Those for which the relationship was significant (Table 4) only at low N offer are indicated with gray boxes. NS indicates that the heterosis value was not significant (Table 2). $IPARi_{PM}$, intercepted incident photosynthetically active radiation during the whole cycle; PGY, plant grain yield; IPAR, incident photosynthetically active radiation; $fIPAR_{PM}$, fraction of incident photosynthetically active radiation intercepted by canopy at physiological maturity; RUE_{PM}, radiation use efficiency at physiological maturity; HI, harvest index; $IPARi_{CP}$, intercepted incident photosynthetically active radiation during the critical period; LAI_{MAX}, maximum leaf area index; $fIPAR_{MAX}$, maximum fraction of incident photosynthetically active radiation intercepted by canopy; RUE_{CP}, radiation use efficiency during critical period; $IPARi_{GF}$, intercepted incident photosynthetically active radiation during the grain-filling period; $fIPAR_{GF}$, fraction of incident photosynthetically active radiation intercepted by canopy during the grain-filling period; RUE_{GF}, radiation use efficiency during the grain-filling period; $IPARi_{CP}$, intercepted incident photosynthetically active radiation during the critical period; PGR_{CP}, plant growth rate during the critical period; $IPARi_{GF}$, intercepted incident photosynthetically active radiation during the grain-filling period; EGR_{CP}, apical ear growth rate during the critical period; KNP, kernel number per plant; KW, kernel weight.

Unlike studies regarding the contribution of heterosis for HI to tolerance to shade (Liu and Tollenaar, 2009a), in our study heterosis for this trait was similar for both HN and LN treatments (31 and 28%, respectively). This differential response deserves several considerations. On one hand it could be attributed to differences in timing and rate of development of stress, because N stress in this study developed slowly and steadily during the vegetative phase, allowing the plants to adequate their size to N availability and affecting biomass production mostly through leaf area expansion (Uhart and Andrade, 1995). Grain yield and total biomass production of both groups of genotypes was affected in a similar way, yielding almost no variation in heterosis for HI across N levels. In contrast, the imposition of shading or water stress during the critical period bracketing silking, once canopy size is almost established, does not allow the plant to adjust its size to resource availability (i.e., to reduce potential growth). The immediate consequence of this type of stress is to decrease assimilate allocation to the developing ear and kernel set dramatically (Boyle et al., 1991; Bolaños and Edmeades, 1993b), and in turn HI (Bolaños and Edmeades, 1993a). Inbreds seemed to be more susceptible

than hybrids to these negative effects of water deficit (Echarte and Tollenaar, 2006) or shading (Liu and Tollenaar, 2009a), which may result in the reported increase in heterosis for HI under these types of stresses. On the other hand, both crowding (Liu and Tollenaar, 2009b) and reduced N supply effects usually start early in the cycle (Uhart and Andrade, 1995; Maddonni et al., 2001), so in these cases differences between treatments in the response of heterosis for HI cannot be attributed a priori to alleged differences in timing and rate of development of stress. The only plausible explanation is that the treatments differed in the explored plant biomass range. According to the intensity of the stress this range may or may not have included the threshold value for plant biomass associated which a drastic decline in HI of each genotype (Echarte and Andrade, 2003). For experiments affected only by LN in this research, the plant biomass range was above this threshold value, therefore no pronounced change should be expected in HI of each genotype (Passioura, 2006) and heterosis should remain stable. For conditions below the critical range for plant biomass (plots at HN affected by severe water deficit in this research, discussed later), HI of all genotypes may be at its lowest value, therefore no significant heterosis for this trait may be expected.

Under N stress alone, the better performance of hybrids relative to their parental inbred lines in grain yield was strongly associated with heterosis for KNP, $Biomass_{PM}$, RUE_{GF}, HI, and traits related to light capture around silking and in less degree to reproductive capacity, irrespective of N supply. This supports the idea that grain yield heterosis reflects cumulative influences of heterosis for many ecophysiological related traits (Griffing, 1990; Tollenaar et al., 2004), which are summarized in Fig. 4. Hybrids produced more grain in proportion to their improved biomass than inbred lines as a result of their enhanced KNP. This grain yield component depends on assimilate supply around silking (Schussler and Westgate, 1994; Westgate et al., 2004), which in our N-conditioned environments was primarily associated with LAI_{MAX} that modulated $fIPAR_{MAX}$ (Fig. 4). Additionally, the high correlation found between heterosis for PGY and heterosis for RUE_{GF} at all N levels supports previous findings on the importance of improving postsilking photosynthetic capacity for increasing maize grain yields (Tollenaar et al., 2004; Tollenaar and Lee, 2006). Changes in relationships between heterosis for physiological traits and grain yield under LN compared to HN, however, were observed. Under LN stress, heterosis for PGY was also correlated with heterosis for RUE_{CP} and KW (Fig. 4) as well as with ASI. The dependence on N availability of the response of PGY heterosis to KW heterosis may be linked to the high response of maize KW to poor growing conditions after kernel set (Borrás et al., 2004), which can affect biomass production during grain filling and grain yield markedly.

Comparing the responses of the F_1 progeny to N supply we observed, on the one hand, that hybrids involving B100 displayed high overall heterosis, although their specific response to N availability depended on the other inbred line included in the cross. On the other hand, hybrids not including B100 as a parent displayed variable overall heterosis regardless of N availability. In other words, the expression of high heterosis did not depend exclusively on the use of B100 as a parental inbred, but its inclusion in the F_1 cross guaranteed a high overall heterosis. This response can be attributed to the greater genetic distance (Springer and Stupar, 2007) between the parental inbred line of United States origin and the Argentine flint germplasm. Contrarily, reduced overall heterosis of most crosses made with LP561 may be attributed to the general poor genetic background for yield determining traits of tropical germplasm, except for the cross with B100 grown at LN conditions. The high heterosis for PGY observed for B100 × LP561 in these environments may result from the combination of high N uptake of LP561 and high N utilization efficiency of B100 reported in previous studies (D'Andrea et al., 2009). This response is in agreement with reports from Presterl et al. (2002), who found both N uptake and N utilization efficiency to be important for improved performance under low N supply. Whereas under high N availability B100 × LP2 showed the highest response to heterosis for PGY compared to the other two B100 derived hybrids. This may be the result of B100 and LP2 inbred lines and their derived hybrid displaying high N utilization efficiency (D'Andrea et al., 2009). Differences in the ability to utilize N accumulated in the plant are responsible of the genotypic variation observed in grain yield at high levels of N supply (Kamprath et. al., 1982).

Heterosis and Combined Abiotic Stresses

Heterosis for PGY and its main determinant (KNP) increased when heat stress took place during the critical period around silking. The main difference in the general pattern described in Fig. 4 was for the shift in heterosis significance of $IPARi_{CP}$ and RUE_{CP}. The former disappeared and the latter turned highly important under heat stress, independently of N level. Reports from heat stress studies on grain yield determination of cereal crops agreed on the large negative effects of this constraint on RUE (Reynolds et al., 2007; Cicchino et al., 2010), which may suppress the effect of differences related to light capture. Our results suggest that the photosynthetic capacity of inbreds may be more affected than that of hybrids under heat stress events during the critical period, independently of well-known negative effects of heat on pollen viability and kernel set (Herrero and Johnson, 1980).

When water stress was severe it became the most limiting factor to grain yield. Moreover, HN supply may have increased the sensitivity of all genotypes to water stress during the critical period because of enhanced vegetative growth at high N availability, which resulted in almost complete depletion of soil water before flowering with the concomitant negative effects on reproductive growth and harvest index (Passioura, 2006). However, the combination of water stress

and HN supply had a greater negative effect for hybrids than inbreds, despite the reported large sensitivity of the latter to drought (Betran et al., 2003). Consequently, when drought occurred mean heterosis for PGY was slightly higher at LN than at HN, and overall heterosis was reduced compared to individual N stress, indicating interactive effects of N and water availabilities. This response may be attributed to the greater responsiveness to N supply of hybrids as compared to inbreds, particularly in tissue expansion (i.e., LAI_{MAX}) that may have enhanced the mentioned detrimental effects of water shortage. In these conditions, the differential response in heterosis for PGY was due to a reduction in hybrid advantage at HN rather than to an increase at LN.

CONCLUSIONS

Our results indicate that changes in heterosis for grain yield with stress highly depend on the nature of the environmental stress; that is, the particular combination of timing, intensity, and development of the stress, or the co-occurrence of stresses. These aspects may affect the relative contribution of the physiological determinants of grain yield to the expression of its heterosis, because of the differential stress sensitivity of each genotype group (i.e., hybrids and inbreds). In our research, heterosis decreased under abiotic restrictions that had a predominant effect of reducing tissue expansion, as for N or water deficits during active vegetative growth before silking. Contrary to many reports, heterosis for PGY was higher under HN than LN, and heterosis for HI was not the main physiological component underlying this differential expression of heterosis for PGY. In agreement with previous findings, the better performance of hybrids relative to their parental inbred lines in grain yield was strongly associated with heterosis for kernel number per plant (KNP), overall biomass production ($Biomass_{PM}$) and its partitioning (HI), sustained photosynthetic capacity during grain filling (RUE_{GF}), and traits related to light capture around silking, irrespective of N supply. Few additional traits (RUE_{CP} and KW) became also important under reduced N availability. The specificity of the response of hybrids to N supply was highly dependent on the combination of parents. Further studies regarding parent progeny relationships and experimental mating designs that allow the analysis of the genetic basis of heterosis in the F_1 progeny are necessary for addressing several issues raised from current research.

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