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Ecophysiological traits in maize hybrids and their parental inbred lines: Phenotyping of responses to contrasting nitrogen supply levels

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ABSTRACT

Maize (*Zea mays* L.) breeding based primarily on final grain yield has been successful in improving this trait since the introduction of hybrids. Contrarily, understanding of the variation in ecophysiological processes responsible of this improvement is limited, especially between parental inbred lines and their hybrids. This limitation may hinder future progress in genetic gain, especially in environments where heritability estimation is reduced because grain yield is severely affected by abiotic stresses. The objective of this study was to analyze the genotypic variation between inbred lines and derived hybrids in the physiological determinants of maize grain yield at the crop level, and how differences among hybrids and parental inbreds may effect contrasting responses to N stress. Special emphasis was given to biomass production and partitioning during the critical period for kernel number determination. Phenotyping included the evaluation of 26 morpho-physiological attributes for 6 maize inbred lines and 12 derived hybrids, cropped in the field at contrasting N supply levels (N_0: no N added; N_{400}: 400 kg N ha^{-1} applied as urea) during three growing seasons. Tested genotypes differed in the response to reduce N supply for most measured traits. Grain yield was always larger for hybrids than for inbreds, but N deficiency affected the former more than the latter (average reduction in grain yield of 40% for hybrids and of 24% for inbreds). We also found (i) a common pattern across genotypes and N levels for the response of kernel number per plant to plant growth rate during the critical period, (ii) a reduced apical ear reproductive capacity (i.e., kernel set per unit of ear growth rate) of inbreds as compared to hybrids, (iii) similar RUE during the critical period and N absorption at maturity at low N levels for both groups of genotypes, but enhanced RUE and N absorption of hybrids at high N supply levels, and (iv) an improved N utilization efficiency of hybrids across all levels of N supply. Results are indicative of a more efficient use of absorbed N by hybrids than by parental inbreds. Larger grain yield of hybrids than of inbreds at N_0 was associated to (i) enhanced dry matter accumulation due to improved light interception during the life cycle and (ii) enhanced biomass partitioning to the grain.

1. Introduction

Maize grain yield has increased steadily since the introduction of hybrids, first in the USA and next in the rest of the world (Duvick, 2005). This trend can be attributed to both breeding and agronomic management practices, in variable proportions depending upon the study (Russell, 1984). There are, however, evidences of strong interaction effects (Tollenaar and Lee, 2002). For example, the analysis of grain yield progress during the last decades in some areas of the USA suggests differences among farming systems. Lack of significant grain yield variation under optimum growing conditions, represented by yield-contest winners at several corn-belt states, indicates no breeding effects on maize potential grain yield after the massive adoption of single-cross hybrids and this potential grain yield has stabilized in a ceiling of ca. 20 Mg ha^{-1} (Tollenaar and Lee, 2002; Cassman et al., 2003). The opposite is evident for other growing conditions, where resource availability (e.g., dryland farming) or resource capture by plants determine a variable level of stress during the crop cycle, with the concomitant penalty on yield potential. Breeding has been successful in keeping a continuous increase in grain yield along the last decades, evidence of the accumulation of favorable alleles (i.e., additive effects characteristic of quantitative traits) conferring enhanced performance under stress conditions (Lee and Tollenaar, 2007). Nevertheless, the understanding of the variation in physiological traits behind these responses is limited, especially between parental inbred lines and derived hybrids. This condition may limit breeding progress in the future, particularly in

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environments where estimates of heritability are reduced due to abiotic stress (Bänziger et al., 1997). A similar consideration applies to the effective application of molecular tools, highly dependent on a correct phenotyping (Tuberosa et al., 2007).

In the development of maize hybrids, gains in heterosis (i.e. improvement of progeny performance respect to mean-parent performance) weakly explain the gain registered for a given trait. Non-heterotic gains, measured as improved inbred line performance, have represented the most important contribution (Duvick, 2005). Consequently, even though the correct combination of inbreds is always a critical step in the production of new hybrids by means of enhanced heterotic effects, efforts for improving inbred line performance per se are justified because they also turn into enhanced hybrid performance. Additionally, improved inbreds allowed for reduced efforts (and costs) in seed production.

Inbred line selection is strongly based on indirect criteria, due to the relatively low correlation for grain yield between inbreds and their derived hybrids; the performance of the former is not a good predictor of final hybrid output (Hallauer and Miranda, 1988; Betrán et al., 2003c). Most part of research on this topic and methods used in commercial maize breeding have been based on morphological traits of easy qualitative detection (e.g., plant vigor, disease resistance, stay-green) or quantification (plant and ear height, time to flowering, percent lodging, grained ears per plant, grain yield, grain composition), due to difficulties for implementing other type of measurements in a large breeding program (Balko and Russell, 1980; Lafitte and Edmeades, 1995; Bänziger and Lafitte, 1997). On one hand, the use of morphological traits, usually measured at flowering or final harvest, is justified by the high correlation detected for them between parental inbreds and their hybrid progeny (Betrán et al., 2003c), in spite of the extremely empiric relationship these traits have with the physiological determinants of grain yield (Tollenaar et al., 2004). On the other hand, there is very little information about differences between hybrids and inbreds in attributes related to the physiological determinants of grain yield; e.g., light interception, radiation use efficiency (RUE), and biomass partitioning represented in harvest index (HI: proportion of final shoot biomass harvested as grain). These traits help quantify final grain yield in a more functional way than do morphological traits listed above. Information on genotypic variation of physiological attributes could optimize the use of inbred lines as descriptors of their hybrids, assisting breeders in the early rejection of poor parents, and consequently, reducing the time and costs of testing a large number of derived hybrids (Betrán et al., 2003a).

Under optimum growing conditions, differences in grain yield between parental inbred lines and their hybrids are mostly determined by the improvement in physiological traits related to (i) light interception, like enhanced leaf area index (LAI) and its persistence along grain filling and HI (Tollenaar et al., 2004) and (ii) photosynthetic capacity during grain filling (Ahmadzadeh et al., 2004). Similar information from resource-limited environments is lacking. Working with only three inbred lines and two derived hybrids under water-deficit and crowding stress (i.e., interplant competition), Echarte and Tollenaar (2006) determined that hybrids had a greater kernel number per plant (KNP) per unit plant growth rate during the critical period around flowering (PGR_{CP}) at all resource levels. There were, however, important differences between hybrids and inbreds for this relationship, which was not strong for all inbreds. This feature deserves further analysis for the correct interpretation of variations in heterosis for kernel set, including a larger set of genotypes than in previous studies, and other resource restrictions. For example, water-availability thresholds that limit tissue expansion due to reduced cell turgor under water-deficit (Ben Haj Salah and Tardieu, 1997; Reymond et al., 2003) may be expected to be independent of plant size, and consequently of inbreeding effects determinant of already reduced growth of inbreds. Contrarily, reduced N supply may limit growth of hybrids more than of inbreds (D'Andrea et al., 2006), because growth restrictions imposed by inbreeding per se determine a reduced nutrient demand for the latter. This difference in growth potential inherent to each group of genotypes may determine a variation between them in plant and soil N contents for the manifestation of stress (i.e., threshold values). Accurate description of these responses is indispensable for a correct interpretation of heterotic effects across N-limited environments.

The general objective of this work was to analyze the genotypic variation between inbred lines and derived hybrids in the physiological determinants of maize grain yield at the crop level, and how differences among hybrids and parental inbreds may effect contrasting responses to N stress. The specific objectives were to study biomass production during the critical period around flowering, its allocation to ear growth and reproductive capacity (i.e., kernel set per unit plant and ear growth rates), because of the well-known response to these variables of the main determinant of grain yield (i.e., final kernel number) under optimum (Andrade et al., 1999) and growth-limited conditions (Andrade et al., 2002; Echarte and Tollenaar, 2006; D'Andrea et al., 2008a). We analyzed differences in mentioned traits among 6 maize inbred lines and 12 derived hybrids cropped at contrasting soil N supply levels. Inbreds representative of different genotypic types were included in the analysis (D'Andrea et al., 2006).

2. Materials and methods

2.1. 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 chosen from a set of 12 inbreds previously phenotyped by D'Andrea et al. (2006, 2008b), which presented variability in breeding eras, origin, canopy size, grain yield and grain yield components, and yield stability across environments. Inbreds also differ in the heterotic group of origin, B100 is US dent germplasm and the rest of the inbreds belong to Argentine flint germplasm. Hybrids included in this study were B100 × LP2, B100 × ZN6, B100 × LP561, ZN6 × LP561, ZN6 × LP611, LP561 × LP662, and all reciprocal crosses.

Paired rows of all six inbreds combinations were sown during 2001–2002, and all possible crosses (i.e., 30 hybrids) were made from them (Stuber, 1980). For ensuring pollen availability for all combination of inbreds (direct and reciprocals), three sowing dates were performed at 3-day intervals during 9 days. The site was fertilized with 400 kg N ha^{-1} supplied as urea in four applications, one at sowing and the rest between V_4 and V_9 (Ritchie and Hanway, 1982). All applications were incorporated to the soil mechanically.

Apical (E_1) and subapical ears of all plants were bagged a few days before silking. Apical ears were pollinated on day 4 after silking with fresh pollen from the corresponding donor inbred, and covered quickly for avoiding contamination with foreign pollen. Subapical ears remained covered permanently and were not used for grain production. Pollination was performed manually between 900 and 1230 h, using fresh pollen from tassels bagged on the previous afternoon. All grained E_1 were harvested at physiological maturity (Daynard and Duncan, 1969), and dried at ambient temperature until kernels reached ca. 12% moisture. Ears were hand-shelled and grain stored in chamber at 8 °C in plastic bags hermetically closed.

2.2. Crop husbandry, experimental design and statistical analysis

Field experiments were conducted at the Pergamino station (33°56′S, 60°34′W) of the National Institute of Agricultural

Technology (INTA), during 2002–2003, 2003–2004 and 2004–2005. Sowing took place on 1-November, 9-October, and 8-November, respectively. The site is representative of the main maize producing area of Argentina (Hall et al., 1982), with soils of the Typic Argiudol group and more than 30 years of continuous agriculture. The top soil (0–40 cm layer) had a pH (water) of 7.3, and an organic matter content of 22 (2002–2003 and 2003–2004) and 14 g kg^{-1} (2004–2005). Mean mineral P content was 86 mg kg^{-1}, and inorganic N at sowing was 55 (2002–2003), 23 (2003–2004) and 40 g kg^{-1} (2004–2005).

Treatments were a factorial combination of 18 genotypes (6 inbreds and 12 single-cross hybrids), and two N levels (N_0: control with no added N; N_{400}: fertilized with 400 kg N ha^{-1}, supplied as urea in four applications between sowing and V_9, as explained above). The experimental design was a split plot, with N supply in the main plot, genotypes in the subplot (hereafter termed plots), and three replicates. Inbreds and hybrids were randomized in the subplot.

The model described in Eq. (1) was used for the combined interannual analysis of data.

$$Y_{ghij} = \mu + \left(\frac{\rho}{\delta}\right)_{gj} + \alpha_h + (\alpha\delta)_{hj} + \left[\left(\frac{\rho}{\delta}\right)\alpha\right]_{gjh} + \beta_i + (\beta\delta)_{ij} + \left[\left(\frac{\rho}{\delta}\right)\beta\right]_{gji} + (\alpha\beta)_{hi} + (\alpha\beta\delta)_{hij} + \left[\left(\frac{\rho}{\delta}\right)\alpha\beta\right]_{ghij} \quad (1)$$

where μ is the grand mean; $(\rho/\delta)_{gj}$ is the effect of the block g nested within the year j; α_h is the effect of the level of N h, and h = 1, 2; $(\alpha\delta)_{hj}$ is the effect of the interaction between the level of N and the year; $[(\rho/\delta)\alpha]_{gjh}$ is the error (a); β_i is the effect of genotype i, and $i = 1, \ldots, 18$; $(\beta\delta)_{ij}$ is the effect of the interaction between the genotype and the year; $[(\rho/\delta)\beta]_{gji}$ is the error (b); $(\alpha\beta)_{hi}$ is the effect of interaction between the level of N and the genotype; $(\alpha\beta\delta)_{hij}$ is the effect of interaction between the level of N, genotypes and years; $[(\rho/\delta)\alpha\beta]_{ghij}$ is the error (c).

The PROC GLM procedure of SAS v 8.2 (SAS Institute, 1999) was used for the ANOVA of each attribute across years. When main or interaction effects were significant ($P < 0.05$), a t-test was used for comparisons among means. Partitions and orthogonal contrasts were used to test differences between direct and reciprocal hybrids and between types of genotypes (i.e., inbreds and hybrids). Regression analysis was applied to the relationship between attributes, and an F-test was used for comparison of slopes and ordinates between fitted models (Prism 4, 2003).

Each plot had three (inbreds) or four (hybrids) rows, of 7 m length and 0.7 m between rows. Plots of inbreds were always separated from those of hybrids by an additional row of the latter. This row was cut and never exceeded 1-m plant height for avoiding an excess of shadow on inbreds. Plots were hand planted at a rate of three seeds per hill, and thinned to one plant per site at V_3. Final stand density was always 7 plants m^{-2}. Water stress was prevented by means of sprinkler irrigation, with the uppermost 1 m of soil held near field capacity throughout the growing season. Crops were kept free of weeds, pests and diseases.

2.3. Measurements

Five successive plants were tagged at V_3 on a central row of each plot to follow leaf appearance, senescence dynamics, and flowering events. Tags were placed at identified positions along the stem (e.g., between leaves 3 and 4), which allowed the identification of individual leaves and the determination of total leaf number (TLN). 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 computed on all tagged plants as lamina length × maximum width × 0.75 (Montgomery, 1911). Prior to silking, leaf area per plant was calculated as the sum of the areas of green ligulated leaves plus the final area of the following two leaves (Muchow and Carberry, 1989). After silking, it was measured as the sum of the area of all green leaves. Leaf area index (LAI) was calculated as the product of leaf area per plant and number of plants per unit land. Hourly recorded values of incident solar radiation and air temperature were obtained at the experimental site with a LI-COR 1200 (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 (PAR) by multiplying by 0.45 (Monteith, 1965), and accumulated thermal time (in °C day with base temperature of 8 °C) was computed from mean daily air temperatures from sowing onwards (Ritchie and NeSmith, 1991). The fraction of incident PAR intercepted by the canopy (fIPAR) was measured fortnightly from V_5 onwards, using a line quantum-sensor (LI-191SA, LI-COR, Lincoln, NE). Four determinations per plot were taken at midday, between 1130 and 1430 h, on clear days, with 1 m of the sensor placed diagonally across a central row and immediately below the lowermost green leaves of the canopy (Gallo and Daughtry, 1986). Daily fIPAR values were obtained by linear interpolation between successive measurements, and the daily amount of intercepted incident PAR was computed as the product between fIPAR and incident PAR. Cumulative incident PAR intercepted values and radiation use efficiency (RUE, estimated as the quotient between biomass production and cumulative incident PAR intercepted) were obtained for the critical period for kernel set between ca. V_{14} and R_2, and for the whole cycle.

Anthesis date (i.e., at least one extruded anther visible at the tassel) and silking date (i.e., at least one silk visible after extruded from the husks) of E_1 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). The latter were used for the computation of thermal time requirements up to anthesis and silking.

Mean values of plant growth rate (PGR_{CP}; in g day^{-1}) and ear growth rate (EGR_{CP}; in g day^{-1}) during the critical period for kernel set (i.e., between the start of active ear growth at ca. V_{14} and the start of active grain filling at R_2; Westgate et al., 2004) were estimated indirectly by means of allometric models. This is widely tested, well documented approach (Vega et al., 2000; Borrás and Otegui, 2001) that has been applied to hybrids and inbreds growing under different abiotic stress conditions (D'Andrea et al., 2006, 2008a; Echarte and Tollenaar, 2006), and is especially valuable for avoiding bulky biomass samples derived from a large number of treatments. The morphometric variables used were plant height from ground level to the uppermost visible ligule and stem diameter at the base of the plant (at ca. V_{14} and R_2), and maximum apical ear diameter (only at R_2). All relationships were highly significant ($P < 0.001$), and no difference was detected in model parameters between reciprocal hybrids. The quotient between mean EGR_{CP} and mean PGR_{CP} (i.e., biomass partitioning ratio around silking) was computed for each treatment combination. Crop growth rate during the critical period (CGR_{CP}; in g m^{-2} day^{-1}) was obtained as the product between mean PGR_{CP} and stand density.

All tagged plants were individually harvested at physiological maturity, i.e. when the black layer was observed in grains of the mid portion of the ear (Daynard and Duncan, 1969) in ears sampled from border rows from 40 days after silking onwards. Each plant was separated into leaf blades, stem plus tassel and sheaths, husks, and ears, and all plant material was oven dried at 60 °C for 7 days and weighed for biomass determination. Grained ears were individually hand-shelled and kernel number was counted for each ear. Kernel number per plant (KNP) was calculated by adding the kernels counted in the apical ear (KN_{E1}) and the subapical ear (when present). For each treatment combination we computed

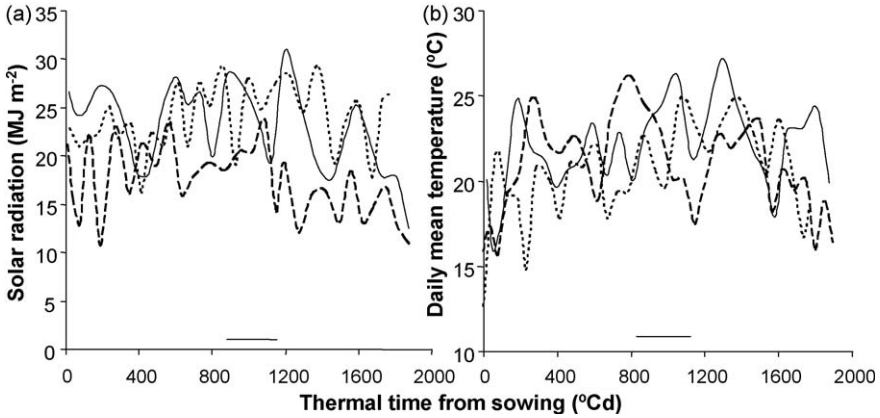

Fig. 1. Solar radiation (a) and mean air temperature (b) evolution during three growing seasons (2002–2003, solid line; 2003–2004, dotted line; 2004–2005, slashed line). Data are presented as a function of thermal time from sowing (in °C day; base temperature of 8 °C). The horizontal line indicates the mean flowering period of experiments.

mean values of (i) harvest index (HI), as the ratio between plant grain yield (PGY) and total shoot plant biomass, (ii) kernel weight (KW), as the ratio between PGY and KNP, (iii) plant reproductive capacity, as the ratio between KNP and PGR_{CP}, and (iv) apical ear reproductive capacity, as the ratio between KN_{E1} and EGR_{CP}.

N concentration was determined for vegetative tissues (leaves, stem, husks and cob) and grains of each plant harvested at maturity in experiments managed during 2002–2003 and 2003–2004. Micro-Kjeldahl analysis was used for the vegetative fraction, and near infrared transmittance (Infratec, 1227, Tecator, Sweden) for the grain fraction. N content (in g plant^{-1}) of each fraction was obtained as the product between N concentration and the corresponding dry weight, and these contents added to give plant N uptake at physiological maturity (in g plant^{-1}). N utilization efficiency (NUE) was computed as the quotient between PGY and plant N uptake.

3. Results

3.1. Weather conditions

Meteorological conditions differed among experimental years (Fig. 1). In general, mean incident solar radiation was similar for 2002–2003 and 2003–2004 seasons (23.2 and 23.9 MJ m^{-2} day^{-1}, respectively), but records indicated a 35% reduction during 2004–2005 season (17.5 MJ m^{-2} day^{-1}). This difference was more remarkable during the grain-filling period, for which mean incident solar radiation during 2004–2005 (15.1 MJ m^{-2} day^{-1}) was 47% smaller than in the other experiments (Fig. 1a). Mean and maximum air temperatures along the cycle did not differ so notably among experiments, and were higher during 2002–2003 (22.2 and 30.4 °C, respectively) than during 2003–2004 (20.8 and 28.2 °C, respectively) and 2004–2005 (21.1 and 28.0 °C, respectively). Differences in temperature accentuated during the critical period (Fig. 1b), mainly because of 11 days (30% of the period) with maximum records above 35 °C in the first season. There were only 3 days (8% of the period) with such a condition in the latest experiment and no record above 35 °C during this period during 2003–2004. These differences between experiments were evident as significant year effects for almost all measured traits, as described next.

3.2. Phenology and light capture

Traits related to crop phenology (TLN, thermal time at anthesis and silking, ASI) and light capture (LAI, fIPAR, intercepted incident

Table 1
Mean squares from the ANOVA of traits related to phenology and light capture.

Source of variation	df[a]	Mean squares[b]									
		Thermal time (°C day)		ASI (days)	TLN	LAI		fIPAR		IPARi (MJ m^{-2} day^{-1})	IPARi (MJ m^{-2})
		Anthesis	Silking			Maximum	PM	Maximum	PM	CP	PM
Y	2	196,376***	246,892***	20.2**	71.37***	4.46***	6.26**	0.29***	0.08 ns	50.84***	460,906***
rep(Y)	6	1,532	1,211	2.9	1.80	0.16	1.01	0.02	0.05	3.12	29,589
N	1	17,547***	92,497*	135.7 ns	0.40 ns	81.60***	209.93***	1.25***	4.87***	126.36 ns	1,548,693**
Y × N	2	576 ns	10,785***	18.1***	0.18 ns	0.73*	0.16 ns	0.20***	0.01 ns	19.14***	87,314***
Error A	6	725	882	1.0	0.31	0.18	0.40	0.004	0.01	0.50	9,315
G	17	53,754***	85,086***	54.8***	14.71***	8.04***	9.46***	0.14***	0.30***	13.96***	160,162***
L vs. H	1	715,068***	867,047***	59.2***	18.61***	89.11***	69.12***	1.87***	2.61***	206.58***	2,123,676***
L	5	14,386***	55,499***	106.0***	22.14***	4.00***	9.80***	0.08***	0.45***	3.89***	74,116***
H	11	11,529***	27,447***	31.2***	10.98***	2.51***	3.88***	0.01**	0.02 ns	1.02*	20,772***
DH vs. RH	1	518 ns	8 ns	1.8 ns	0.74 ns	0.01 ns	0.41 ns	0.01 ns	0.01 ns	0.09 ns	14 ns
DH	5	13,107***	30,982***	33.5***	12.58***	3.08***	4.32***	0.0008 ns	0.01 ns	0.89 ns	23,040***
RH	5	12,152***	29,401***	34.7***	11.43***	2.45***	4.14***	0.02***	0.02 ns	1.34**	22,656***
Y × G	34	1,467***	1,903***	3.2***	0.41***	0.23***	0.59***	0.01***	0.03***	0.53**	3,876**
N × G	17	653 ns	1,572*	1.9 ns	0.11 ns	1.04***	5.09***	0.005 ns	0.06***	0.55*	10,723***
N × G × Y	34	317 ns	835 ns	1.7**	0.16 ns	0.14 ns	0.46***	0.003 ns	0.02***	0.33 ns	2,215 ns
Error B	204	484	729	1.0	0.17	0.11	0.26	0.005	0.01	0.36	2,286
Total	323										

[a] df: degrees of freedom, ASI: anthesis–silking interval; TLN: total leaf number; LAI: leaf area index; fIPAR: fraction of intercepted incident PAR; IPARi: cumulative incident PAR; PM: physiological maturity; CP: critical period; Y: year; rep: replicate; N: nitrogen, G: genotype; L: inbred lines; H: hybrids; DH: direct hybrid; RH: reciprocal hybrid.
[b] ns, *, **, *** *F*-test not significant and significant at $P < 0.10$, $P < 0.05$ and $P < 0.01$, respectively.

Table 2
Mean values of traits related to phenology and light capture for 6 inbred lines and 12 derived hybrids.

Genotype	N	Thermal time (°C day)[a]		ASI (day)	TLN	LAI		flPAR		IPARi ($MJ\,m^{-2}\,day^{-1}$)	IPARi ($MJ\,m^{-2}$)
		Anthesis	Silking			Max	PM	Max	PM	CP	PM
B100	N_{400}	999	993	−0.38	19.6	2.55	1.56	0.73	0.36	6.77	468
	N_0	981	978	−0.20	19.8	2.40	0.89	0.66	0.24	6.34	417
LP2	N_{400}	1001	1051	3.54	22.0	2.99	0.23	0.76	0.14	7.54	443
	N_0	1011	1066	3.91	22.0	2.57	0.51	0.54	0.15	5.62	358
ZN6	N_{400}	984	1002	1.34	19.9	2.73	1.95	0.65	0.49	6.23	473
	N_0	988	1010	1.55	19.8	2.34	1.12	0.56	0.30	5.34	384
LP561	N_{400}	1009	1079	5.13	19.4	2.98	2.39	0.71	0.57	7.04	547
	N_0	1011	1098	6.76	19.2	2.68	1.96	0.58	0.46	5.52	451
LP662	N_{400}	970	991	1.32	19.1	3.61	1.94	0.76	0.55	7.40	581
	N_0	998	1039	2.92	19.2	2.94	1.08	0.64	0.34	6.12	444
LP611	N_{400}	1049	1109	4.69	19.1	4.06	3.20	0.82	0.68	7.76	629
	N_0	1069	1153	6.30	19.1	3.31	2.19	0.72	0.57	6.83	553
B100 × LP2[b]	N_{400}	896	908	0.85	21.7	4.70	2.62	0.94	0.68	9.57	695
	N_0	912	934	1.48	21.7	3.39	1.64	0.80	0.44	7.83	559
B100 × ZN6	N_{400}	873	864	−0.55	20.1	4.27	3.05	0.91	0.75	8.92	712
	N_0	889	903	0.87	20.2	3.14	1.42	0.78	0.45	7.69	544
B100 × LP561	N_{400}	884	899	1.01	19.8	4.40	3.75	0.91	0.77	8.98	748
	N_0	901	936	2.27	19.8	3.31	1.10	0.77	0.40	7.50	569
ZN6 × LP561	N_{400}	897	931	2.35	20.1	4.51	4.05	0.90	0.79	8.89	767
	N_0	909	970	4.10	19.9	3.27	1.37	0.75	0.42	7.39	568
ZN6 × LP611	N_{400}	945	978	2.45	21.1	5.72	5.35	0.93	0.86	9.44	839
	N_0	969	1029	4.24	20.8	3.87	1.71	0.81	0.47	7.95	623
LP561 × LP662	N_{400}	887	924	2.42	19.6	4.52	4.02	0.90	0.77	8.74	764
	N_0	911	978	4.46	19.7	3.39	1.68	0.77	0.45	7.53	586
Inbreds mean	N_{400}	1002	1038	2.61	19.9	3.15	1.88	0.74	0.47	7.12	524
	N_0	1010	1057	3.54	19.8	2.71	1.29	0.62	0.34	5.96	434
Hybrids mean	N_{400}	897	917	1.42	20.4	4.69	3.81	0.92	0.77	9.09	754
	N_0	915	958	2.90	20.3	3.40	1.49	0.78	0.44	7.65	575
LSD G × N ($P<0.05$)[c]		22	27	0.99	0.41	0.33	0.51	0.07	0.10	0.60	48

[a] ASI: anthesis–silking interval; TLN: total leaf number; LAI: leaf area index; flPAR: fraction of intercepted incident PAR; IPARi: cumulative IPARi; Max: maximum; PM: physiological maturity; CP: critical period; N_0: control with no N added; N_{400}: fertilized with 400 kg N ha^{-1}.
[b] Mean of direct and reciprocal hybrids due to lack of significant differences between reciprocal crosses (see ANOVA Table 1).
[c] Least significant difference ($P<0.05$) for genotype × nitrogen (G × N) interaction effects indicates the difference between any combination of genotype and N level.

PAR) differed among genotypes ($P<0.01$; Table 1). On average, cycle duration up to flowering (i.e., thermal time requirements to reach anthesis and silking) was shorter for hybrids than for inbreds (Table 2) in spite of similar TLN of most genotypes (Table 2). Likewise, hybrids had a shorter ASI than inbreds (Table 2). Contrarily, traits related to canopy size (maximum LAI) and light capture efficiency (maximum flPAR and flPAR at physiological maturity) were larger for the former than for the latter (Table 2). Therefore, the differences in cycle duration mentioned were overcompensated with the concomitant increase in the amount of intercepted PAR of hybrids as compared to inbreds (Table 2). A significant ($P<0.05$) variation was detected for all these traits among genotypes within each group, but not between direct and reciprocal hybrids (Table 1).

Nitrogen deficiencies did not modify TLN (Table 2), but promoted a significant ($P<0.01$) delay in thermal time to anthesis of hybrids ZN6 × LP611 and LP561 × LP662, and of inbred LP662. A similar trend ($P<0.10$) was detected in thermal time to silking of most hybrids and of inbreds LP662 and LP611. The described responses determined a significant ($P<0.05$) increase in ASI under N stress, except for hybrid B100 × LP2. A significant ($P<0.05$) Y × N and Y × G × N was detected for ASI and thermal time to silking (Table 1). These interactions were observed as a significant ($P<0.05$) reduction in thermal time to silking and ASI at N_{400} and a significant ($P<0.05$) increase at N_0 for inbreds LP2 and LP662 and most hybrids in the experiment managed during 2002–2003 in comparison with the others experiments (data not shown).

Canopy size and light capture of most genotypes declined in response to N_0 level (Tables 1 and 2). The only exceptions to this trend were light capture at flowering of inbred B100 and at physiological maturity of inbred LP2, for which no difference was detected between N levels. The magnitude of the reduction was always larger for hybrids than for inbreds (Table 2), for example in (i) maximum LAI (28% for hybrids and 14% for inbreds), (ii) LAI at physiological maturity (61% for hybrids and 31% for inbreds), (iii) flPAR at physiological maturity (43% for hybrids and 26% for inbreds), and (iv) amount of intercepted incident PAR at physiological maturity (34% for hybrids and 17% for inbreds).

3.3. Biomass production and its partitioning

Biomass production along the cycle, RUE during whole cycle, PGR_{CP} and HI were significantly ($P<0.05$) larger for hybrids than for inbreds (Table 3), but mean EGR_{CP} did not differ between these two groups (Table 4). These trends in growth rates (i.e., large variation in PGR_{CP} and similarity in EGR_{CP}) indicated a reduced biomass partitioning ratio to the earshoot (i.e., $EGR_{CP}\ PGR_{CP}^{-1}$) in hybrids (ratio = 0.28) as compared to their parental inbred lines

Table 3
Mean squares from the ANOVA of traits related to biomass production and its partitioning.

Source of variation	df[a]	Mean squares[b]								
		Biomass ($g\,pl^{-1}$)			RUE		PGR_{CP} ($g\,pl^{-1}\,day^{-1}$)	EGR_{CP} ($g\,day^{-1}$)	$EGR_{CP}\,PGR_{CP}^{-1}$	HI
		Pre-CP	Silking + 12 days	PM	CP	PM				
Y	2	10,884***	7,129**	17,807**	6.21***	6.61***	0.33 ns	0.173 ns	0.044***	0.0638***
rep(Y)	6	257	717	2,251	0.42	0.21	0.33	0.069	0.003	0.0006
N	1	16,712*	96,676**	515,680**	20.40**	8.99***	97.04**	5.869*	0.008 ns	0.036 ns
Y × N	2	1,035**	4,138**	17,754***	0.59 ns	0.18 ns	3.93***	0.559***	0.004 ns	0.0294**
Error A	6	150	577	1,507	0.46	0.23	0.36	0.037	0.002	0.0017
G	17	5,616***	7,716***	35,099***	0.68**	0.74***	3.58***	0.386***	0.036***	0.0573***
L vs. H	1	86,755***	98,854***	546,430***	0.88 ns	6.54***	50.27***	0.206**	0.217***	0.4561***
L	5	1,178**	3,001***	6,115***	1.55***	0.90***	0.97 ns	0.761***	0.050***	0.0599***
H	11	257 ns	1,574**	1,788***	0.26 ns	0.13**	0.53 ns	0.216***	0.013 ns	0.0198***
DH vs. RH	1	127 ns	371 ns	165 ns	0.12 ns	0.02 ns	0.28 ns	0.003 ns	0.001 ns	0.0001 ns
DH	5	369 ns	1,892**	2,248***	0.30 ns	0.19**	0.58 ns	0.246***	0.013 ns	0.0228***
RH	5	171 ns	1,497*	1,653***	0.25 ns	0.10 ns	0.53 ns	0.228**	0.014 ns	0.0208***
Y × G	34	462***	658***	610 ns	0.34***	0.08 ns	0.50***	0.070***	0.009***	0.0045***
N × G	17	207***	1,149***	7,201***	0.85***	0.29***	1.28***	0.050***	0.007***	0.0029***
N × G × Y	34	43 ns	313 ns	493 ns	0.25***	0.09*	0.37***	0.020 ns	0.002**	0.0011 ns
Error B	204	97	284	523	0.10	0.07	0.12	0.021	0.001	0.0011
Total	323									

[a] df: degrees of freedom, CP: critical period; PM: physiological maturity; RUE: radiation use efficiency; PGR_{CP}: plant growth rate during the critical period; EGR_{CP}: ear growth rate during the critical period; HI: harvest index; Y: year; rep: replicate; N: nitrogen; G: genotype; L: inbred lines; H: hybrids; DH: direct hybrid; RH: reciprocal hybrid.
[b] ns, *, **, *** *F*-test not significant and significant at $P < 0.10$, $P < 0.05$ and $P < 0.01$, respectively.

Table 4
Mean values of traits related to plant biomass production and its partitioning for 6 inbred lines and 12 derived hybrids.

Genotype	N	Biomass ($g\,pl^{-1}$)[a]			RUE		PGR_{CP} ($g\,pl^{-1}\,day^{-1}$)	EGR_{CP} ($g\,day^{-1}$)	$EGR_{CP}\,PGR_{CP}^{-1}$	HI
		Pre-CP	Silking + 12 days	PM	CP	PM				
B100	N_{400}	31.3	102	151	2.68	2.31	2.56	0.94	0.38	0.36
	N_0	28.9	88	124	2.46	2.06	2.20	0.81	0.37	0.34
LP2	N_{400}	36.4	118	133	2.49	2.16	2.68	1.03	0.39	0.38
	N_0	30.7	97	120	2.71	2.37	2.10	0.77	0.37	0.39
ZN6	N_{400}	36.6	113	161	3.15	2.41	2.74	0.74	0.27	0.31
	N_0	31.1	93	128	2.75	2.34	2.09	0.58	0.28	0.31
LP561	N_{400}	32.9	108	174	2.39	2.24	2.38	0.73	0.31	0.29
	N_0	28.8	106	146	2.91	2.25	2.26	0.61	0.26	0.26
LP662	N_{400}	61.1	158	210	3.30	2.55	3.49	1.45	0.40	0.36
	N_0	42.9	114	158	2.69	2.58	2.30	0.95	0.40	0.32
LP611	N_{400}	39.5	121	169	2.12	1.90	2.29	0.77	0.33	0.24
	N_0	33.2	105	135	2.12	1.76	1.99	0.47	0.24	0.17
B100 × LP2[b]	N_{400}	81.4	176	280	3.11	2.84	4.23	1.17	0.28	0.47
	N_0	61.3	127	188	2.42	2.37	2.67	0.91	0.34	0.42
B100 × ZN6	N_{400}	82.2	172	289	3.50	2.91	4.43	1.12	0.26	0.44
	N_0	65.2	127	183	2.45	2.35	2.67	0.83	0.31	0.42
B100 × LP561	N_{400}	80.3	167	312	3.04	2.93	3.88	1.07	0.28	0.44
	N_0	63.0	129	191	2.59	2.38	2.73	0.79	0.29	0.38
ZN6 × LP561	N_{400}	70.9	151	289	2.83	2.66	3.57	0.82	0.23	0.36
	N_0	56.2	118	183	2.36	2.27	2.46	0.56	0.23	0.35
ZN6 × LP611	N_{400}	81.4	196	331	3.14	2.78	4.26	1.00	0.24	0.35
	N_0	60.9	137	185	2.21	2.12	2.49	0.69	0.28	0.37
LP561 × LP662	N_{400}	82.9	178	310	3.30	2.86	4.09	1.16	0.29	0.41
	N_0	64.2	126	198	2.25	2.37	2.42	0.69	0.28	0.36
Inbreds mean	N_{400}	39.6	120	166	2.69	2.26	2.69	0.95	0.35	0.32
	N_0	32.6	101	135	2.61	2.23	2.16	0.70	0.32	0.30
Hybrids mean	N_{400}	79.8	174	302	3.15	2.83	4.08	1.06	0.26	0.41
	N_0	61.8	127	188	2.38	2.31	2.57	0.74	0.29	0.39
LSD G × N ($P < 0.05$)[c]		9.8	17	23	0.31	0.26	0.34	0.14	0.03	0.03

[a] CP: critical period; PM: physiological maturity; RUE: radiation use efficiency; PGR_{CP}: plant growth rate during the critical period; EGR_{CP}: ear growth rate during the critical period; HI: harvest index; N_0: control with no N added; N_{400}: fertilized with 400 kg N ha^{-1}.
[b] Mean of direct and reciprocal hybrids due to lack of significant differences between reciprocal crosses (see ANOVA Table 3).
[c] Least significant difference ($P < 0.05$) for genotype × nitrogen (G × N) interaction effects indicates the difference between any combination of genotype and N level.

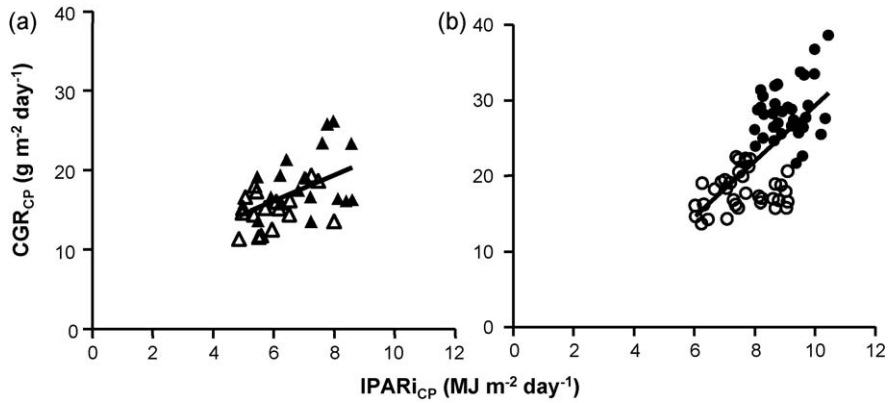

Fig. 2. Relationship between crop growth rate during the critical period (CGR_{CP}) and the amount of incident PAR intercepted per day during the critical period ($IPARi_{CP}$) of 6 inbred lines (a) and 12 derived hybrids (b) cropped during three experimental years at contrasting N levels (with 400 kg N ha^{-1}, N_{400} and with no N added, N_0). Symbols represent different genetic materials (triangles for inbreds and circles for hybrids) and N levels (closed for N_{400} and open for N_0). Fitted models were (a) $CGR_{CP} = 1.62\ IPARi_{CP} + 6.41$ ($r^2 = 0.25$, $n = 36$, $P < 0.01$) and (b) $CGR_{CP} = 3.68\ IPARi_{CP} - 7.54$ ($r^2 = 0.42$, $n = 72$, $P < 0.001$).

(ratio = 0.34), except for inbreds ZN6 and LP561 that did not differ markedly from their hybrid progeny and LP662 that largely overcome the rest of the genotypes. There were, however, significant ($P < 0.05$) differences among genotypes within each group for biomass production and many related traits, except for (i) PGR_{CP} (among inbreds and among hybrids) and (ii) biomass production before silking, RUE during the critical period, and EGR_{CP} PGR_{CP}^{-1} (among hybrids). There were no differences between direct and reciprocal hybrids for these attributes, as indicated previously for those related to phenology and light capture.

For inbreds, there were a significant ($P < 0.05$) reduction in biomass at physiological maturity (17%), PGR_{CP} (20%), and EGR_{CP} (26%) at N_0 supply level. For hybrids, the decline in these traits reached 31, 37, and 30%, respectively. Significant ($P < 0.05$) N × Y, G × Y, and G × N × Y interactions allowed for the detection of differences among genotypes in several traits (Table 3). For instance, N stress promoted a significant ($P < 0.05$) reduction in (i) RUE of hybrids but not of inbreds, (ii) HI of some inbreds (LP561, LP611, and LP662) and hybrids (B100 × LP2, B100 × LP561, and LP561 × LP662), and (iii) the biomass partitioning ratio of some inbreds (LP561 and LP611) but of no hybrid (Table 3).

For all genotypes, variations in light capture (Fig. 2) and RUE during the critical period (Table 4) determined the observed variation in crop growth rate during the critical period (CGR_{CP}). All factors (years, N and genotypes) modulated these responses. Two different models were fitted to the relationship between CGR_{CP} and incident PAR intercepted during the critical period between inbreds (Fig. 2a) and hybrids (Fig. 2b). The slopes of these relationships represented mean RUE during the critical period (1.62 and 3.68 g MJ^{-1} for inbreds and hybrids, respectively). Inbred lines did not differ between high and low N, i.e. most inbred data were below 8 MJ m^{-2} day^{-1} with no clear distinction between N levels. Contrarily, all hybrids grown at N_{400} had values above 8 MJ m^{-2} day^{-1} and most hybrids grown at N_0 were within the inbreds range of the response (i.e., below 8 MJ m^{-2} day^{-1}). Nitrogen did not affect RUE during the critical period for kernel set of inbreds; the variation observed for this trait was due to year and genotype effects but not to contrasting N offers (Table 4). For a given inbred, differences in CGR_{CP} were chiefly due to larger light capture (incident PAR intercepted during the critical period) at N_{400} (mean of 7.12 MJ m^{-2} day^{-1}, Table 2) than at N_0 (mean of 5.96 MJ m^{-2} day^{-1}, Table 2). Among

Table 5
Mean squares from the ANOVA of traits related to grain yield determination and N absorption.

Source of variation	df[a]	Mean squares[b]					df	Mean squares	
		KNP (pl^{-1})	KNP PGR_{CP}^{-1} (grains day g^{-1})	KN_{E1} EGR_{CP}^{-1} (grains day g^{-1})	KW (mg)	PGY (g pl^{-1})		PNU_{PM} (kg ha^{-1})	NUE (kg grain kg N absorbed^{-1})
Y	2	123,350***	18,181***	198,823***	167 ns	6,614***	1	5,773 ns	893***
rep(Y)	6	5,068	130	5,437	499	495	4	2,137	16
N	1	773,020*	3,330*	11,237 ns	83,901*	98,270*	1	864,311***	20,321*
Y × N	2	67,505***	184 ns	7,011 ns	6,172***	8,461***	1	555 ns	463**
Error A	6	3,711	365	3,205	345	294	4	377	39
G	17	85,690***	2,452***	92,078***	16,283***	10,386***	17	6,331***	652***
L vs. H	1	1,131,916***	17,286***	1,192,368***	229,427***	157,195***	1	91,592***	7,550***
L	5	36,363***	3,663***	22,415**	4,894***	1,542***	5	1,803***	322***
H	11	12,999***	554 ns	23,716***	2,083***	1,060***	11	639**	176***
DH vs. RH	1	690 ns	180 ns	741 ns	0.37 ns	44 ns	1	174 ns	5 ns
DH	5	17,242***	544 ns	19,742**	2,854***	1,424***	5	506 ns	156***
RH	5	11,217*	638 ns	32,285***	1,729***	899***	5	864**	229***
Y × G	34	4,689***	911***	7,528***	427***	194**	17	126 ns	26 ns
N × G	17	7,617***	1,006*	6,586 ns	1,329***	1,405***	17	5,850***	155***
N × G × Y	34	2,348 ns	547***	4,805***	377***	168 ns	17	333 ns	34 ns
Error B	204	1,773	177	2,169	215	127	134	298	22
Total	323						213		

[a] df: degree of freedom; KNP: kernel number per plant; PGR_{CP}: plant growth rate during the critical period; KN_{E1}: kernel number per apical ear; EGR_{CP}: ear growth rate during the critical period; KW: kernel weight; PGY: plant grain yield; PNU_{PM}: plant nitrogen uptake at physiological maturity; NUE: nitrogen utilization efficiency; Y: year; rep: replicate; N: nitrogen; G: genotype; L: inbred lines; H: hybrids; DH: direct hybrid; RH: reciprocal hybrid.

[b] ns, *, **, *** *F*-test not significant and significant at $P < 0.10$, $P < 0.05$ and $P < 0.01$, respectively.

Table 6
Mean values of traits related to grain yield determination and N absorption computed for 6 inbred lines and 12 derived hybrids.

Genotype	N	KNP[a] (pl^{-1})	KNP PGR_{CP}^{-1} (grains day g^{-1})	KN_{E1} EGR_{CP}^{-1} (grains day g^{-1})	KW (mg)	PGY ($g\,pl^{-1}$)	PNU_{PM} ($kg\,ha^{-1}$)	NUE (kg grain kg N absorbed^{-1})
B100	N_{400}	255	98	251	215	55.8	137	33.9
	N_0	221	97	255	197	44.6	77	47.5
LP2	N_{400}	308	118	300	165	50.5	136	29.4
	N_0	290	138	372	161	47.2	67	51.0
ZN6	N_{400}	263	97	307	197	52.0	143	29.2
	N_0	210	103	351	190	40.4	74	39.1
LP561	N_{400}	250	107	363	203	50.6	162	25.2
	N_0	192	85	325	195	38.0	76	38.0
LP662	N_{400}	349	101	279	221	76.1	201	28.1
	N_0	269	117	294	201	52.2	86	42.8
LP611	N_{400}	224	96	282	190	41.8	174	20.2
	N_0	137	69	260	168	24.7	67	26.8
B100 × LP2[b]	N_{400}	489	121	427	266	130.4	240	40.5
	N_0	357	135	399	225	81.7	69	73.8
B100 × ZN6	N_{400}	446	103	389	286	126.1	256	36.6
	N_0	349	131	424	221	78.1	75	69.9
B100 × LP561	N_{400}	453	119	434	300	136.0	274	36.4
	N_0	301	110	390	247	74.8	77	61.0
ZN6 × LP561	N_{400}	386	110	491	268	104.0	269	30.3
	N_0	277	114	521	230	64.2	76	56.7
ZN6 × LP611	N_{400}	451	109	452	258	115.6	282	31.6
	N_0	317	129	482	218	68.7	76	63.6
LP561 × LP662	N_{400}	464	118	421	275	127.1	270	35.2
	N_0	304	127	475	239	72.7	77	60.0
Inbreds mean	N_{400}	275	103	297	198	54.5	159	27.7
	N_0	220	101	309	185	41.2	74	40.9
Hybrids mean	N_{400}	448	113	436	276	123.2	265	35.1
	N_0	317	124	449	230	73.4	75	64.2
LSD G × N ($P < 0.05$)[c]		42	13	46	15	11.2	17	4.7

[a] KNP: kernel number per plant; PGR_{CP}: plant growth rate during the critical period; KN_{E1}: kernel number per apical ear; EGR_{CP}: ear growth rate during the critical period; KW: kernel weight; PGY: plant grain yield; PNU_{PM}: plant nitrogen uptake at physiological maturity; NUE: nitrogen utilization efficiency; N_0: control with no N added; N_{400}: fertilized with 400 kg N ha^{-1}.
[b] Mean of direct and reciprocal hybrids due to lack of significant differences between reciprocal crosses (see ANOVA Table 5).
[c] Least significant difference ($P < 0.05$) for genotype × nitrogen (G × N) interaction effects indicates the difference between any combination of genotype and N level.

hybrids, the reduction of 24.4% in mean RUE during the critical period promoted at N_0 (Table 4) was accompanied by 15.8% decline in mean intercepted incident PAR during this same period (Table 2). As a result, RUE of inbred lines and hybrids did not differ at low N, but RUE is greater for hybrids than for inbreds at high N (Table 4). In spite of this general response, some inbreds (e.g., ZN6 and LP662) did not differ from their derived hybrids when grown at N_{400}.

3.4. Grain yield determination

As for most traits related to light capture and biomass production, hybrids had larger grain yield ($P < 0.05$) than their parental inbred lines (Tables 5 and 6), and most part of its variation was explained by variations in KNP ($r^2 = 0.88$, $n = 36$, $P < 0.001$ for inbreds; $r^2 = 0.92$, $n = 72$, $P < 0.001$, for hybrids) rather than by variations in KW ($r^2 = 0.24$, $P < 0.01$, for inbreds; $r^2 = 0.74$, $P < 0.001$, for hybrids). On average, N deficiency caused a decline ($P < 0.10$) in KNP (29% for hybrids and 20% for inbreds) and PGY (40% for hybrids and 24% for inbreds). The decrease in KW at N_0 was significant ($P < 0.05$) only among hybrids (17% reduction). There was a strong ($P < 0.01$) G × N interaction for all these traits (Table 5); for example, KNP of most genotypes decreased markedly ($P < 0.05$) at N_0 as compared to N_{400}, except for inbreds B100 and LP2.

Differences in PGY between inbreds and hybrids were matched by a largely enhanced plant reproductive capacity (KNP PGR_{CP}^{-1}) and apical ear reproductive capacity (KN_{E1} EGR_{CP}^{-1}) of hybrids as compared to inbreds (Tables 5 and 6). There was, however, genotypic variability for KNP PGR_{CP}^{-1} (only among inbreds) and for KN_{E1} EGR_{CP}^{-1} (among inbreds and among hybrids). For the whole data set, a single model accommodated most part of the variation registered in the determinants of plant reproductive capacity ($r^2 = 0.69$, $n = 108$, $P < 0.001$), evidence of a similar response pattern for parental inbreds and derived hybrids independently of N levels (Fig. 3a). This model indicated a threshold PGR_{CP} of 0.91 g $plant^{-1}$ day^{-1} for having any kernel set. At each N level, however, inbreds usually explored the lower ranges of PGR_{CP} and KNP (Tables 4 and 6). Though parental inbreds had a smaller plant reproductive capacity than their derived hybrids (Table 6), this trend was promoted exclusively by the poor performance of LP561, ZN6 and LP611 during 2004–2005 season; contrarily, inbreds LP662 and LP2 had quotient values similar than their derived hybrids. The decline in the quotient for mentioned inbreds during that season was evident in the analysis of the whole data set (Fig. 3a). Their departure from the general model could be attributed to the strong reduction in irradiance registered from 1100 °C day onwards in this experiment (average of 15 MJ m^{-2} day^{-1}) respect to experiments managed during 2002–2003 and 2003–2004 (>20 MJ m^{-2} day^{-1}), which affected

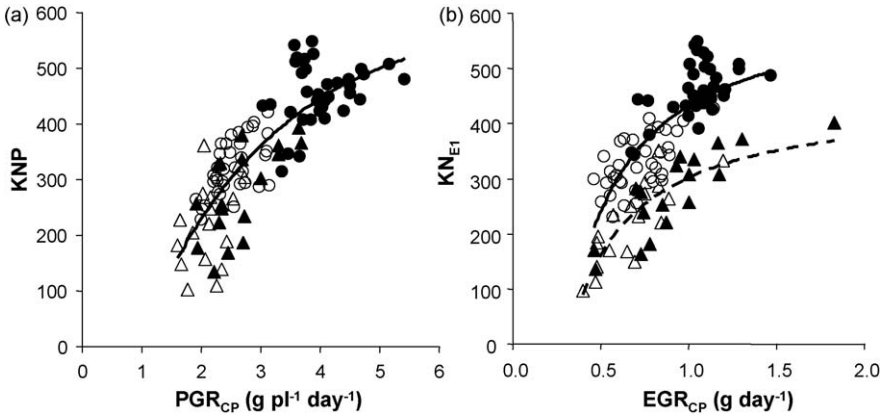

Fig. 3. Relationship between (a) plant growth rate during the critical period (PGR_{CP}) and kernel number per plant (KNP), and (b) ear growth rate during the critical period (EGR_{CP}) and kernel number per apical ear (KN_{E1}) of 6 inbred lines and 12 derived hybrids cropped during three experimental years at contrasting N levels (with 400 kg N ha^{-1}, N_{400} and with no N added, N_0). Symbols as in Fig. 2. Fitted models were (a) KNP = 590{1 − exp[−(PGR_{CP} − 0.91)/2.31]} (r^2 = 0.69, n = 108, $P < 0.001$); (b) KN_{E1} = 609 − 193/EGR_{CP} (r^2 = 0.62, n = 72, $P < 0.001$) for hybrids (solid line), and KN_{E1} = 435 − 144/EGR_{CP} (r^2 = 0.72, n = 36, $P < 0.001$) for inbreds (dotted line).

flowering of the most delayed genotypes (inbreds LP611 and LP561).

In contrast to plant reproductive capacity, two models were necessary for an adequate description of the relationship between variables determinant of apical ear reproductive capacity (i.e., KN_{E1} EGR_{CP}^{-1}), because hybrids tended to set more kernels than inbreds for a given level of EGR_{CP} (Table 6, Fig. 3b).

3.5. N absorption

Hybrids and inbreds differed ($P < 0.01$) in the amount of N absorbed at physiological maturity and in NUE (Tables 5 and 6). N offer also affected these traits ($P < 0.10$), but a significant ($P < 0.05$) G × N interaction was detected for them. Both groups of genetic materials had a similar plant N uptake at physiological maturity when N offer was reduced at N_0, but the increase in this trait at N_{400} was larger for hybrids than for inbreds (Tables 5 and 6). The N_0 level promoted a reduction in plant N uptake at physiological maturity of 53% in inbreds and 72% in hybrids. Contrarily, NUE at N_0 was larger ($P < 0.05$) than at N_{400} for hybrids (64 and 35 kg grain kg N^{-1} absorbed, respectively) and for inbreds (41 and 28 kg grain kg N^{-1} absorbed, respectively). Besides, N recovery (i.e., difference of plant N uptake between N_{400} and N_0 per unit of N applied) estimates differed markedly ($P < 0.01$) between groups of genotypes. These estimates averaged 0.21 and 0.48 kg N absorbed kg N applied^{-1} for inbreds and hybrids, respectively.

Variations in plant N uptake explained the variations observed in PGY (Fig. 4), but different models were fitted to hybrids (r^2 = 0.89, $P < 0.001$) and inbreds (r^2 = 0.52, $P < 0.001$). A nitrogen uptake threshold for plant barrenness (0.28 g N plant^{-1} for inbreds and 0.34 g N plant^{-1} for hybrids) was estimated as the positive intercept of the relationship between PGY and N absorbed at maturity. Derived hybrids always yielded more than parental inbreds at a given level of N absorbed, in agreement with the significant G × N interactions described above.

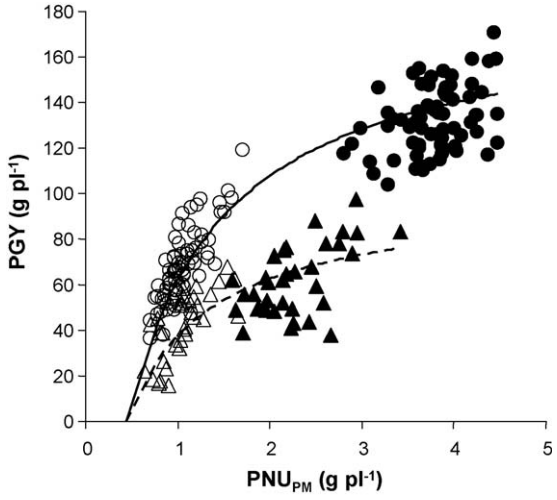

Fig. 4. Relationship between plant grain yield (PGY) and plant N uptake at physiological maturity (PNU_{PM}) of 6 inbred lines and 12 derived hybrids cropped during 2002–2003 and 2003–2004 experiments at contrasting N levels (with 400 kg N ha^{-1}, N_{400} and with no N added, N_0). Symbols as in Fig. 2. Fitted models were PGY = 158{1 − exp[−(PNU_{PM} − 0.34)/0.90]} (r_2 = 0.89, n = 144, $P < 0.001$) for hybrids (solid line), and PGY = 97{1 − exp[−(PNU_{PM} − 0.28)/1.01]} (r^2 = 0.52, n = 72, $P < 0.001$) for inbreds (doted line).

4. Discussion

In this work we focused on genotypic differences between 6 parental inbreds and 12 derived hybrids for 26 morpho-physiological traits evaluated at contrasting N supply levels across three growing seasons. We observed that N stress imposed in this study was not the same for the hybrids and the inbred lines. The high N level represented unlimited N supply for both groups, but the low N level was more stressful for the hybrids than for the inbreds. Tested genotypes differed for most of the evaluated traits, both between and within groups of genotypes. N supply also affected the expression of these traits, except for TLN. This result confirmed previous evidence (Uhart and Andrade, 1995) on maize physiological responses to variable N offer obtained for one commercial hybrid. The existence of significant G × N and G × N × Y interactions for most measured traits, together with the lack of agreement between inbreds and hybrids in the response determined for several relationships between traits, revealed that both genetic materials evaluated were affected differentially by environmental conditions, especially by N supply. These conditions modified the ranking of genotypes, a response indicative of different physiological processes (and consequently genetic controls) behind grain yield determination at contrasting environments offers (Falconer, 1989). The exceptions to this trend were reciprocal crosses of the same hybrid, evidence of no maternal or paternal effects.

Thermal time requirements to reach anthesis were shorter for hybrids than for inbreds, in agreement with previous evidences (Hallauer and Miranda, 1988; Betrán et al., 2003b). This difference, however, was not related to variation in TLN; therefore, both

groups of genotypes differed in leaf appearance rate (Tollenaar and Lee, 2006). Reduced N supply caused a delay in thermal time to flowering, which was negligible for thermal time to anthesis (mean of 13 °C day, i.e. less than 1 day) but important for thermal time to silking (mean of 30 °C day, i.e. between 1 and 3 days). The response of each flowering event to N supply was expected (Edmeades et al., 1993; Lafitte and Edmeades, 1994a), because abiotic stress affects silk growth more than pollen production with the concomitant increase in ASI. For this reason, the variation in ASI has been used many times as a good surrogate of plant or ear growth for the estimation of final kernel number (Bolaños and Edmeades, 1993).

In agreement with most reports from literature (Cirilo and Andrade, 1994; Otegui et al., 1995; Westgate et al., 2004; Fischer, 2008), KNP was the main determinant of PGY, but genotypes differed in plant reproductive capacity (KNP PGR_{CP}^{-1}). The range established for this trait among single-cross hybrids used in the current research (between 109 and 135 KNP PGR_{CP}^{-1}) was within values reported by Echarte et al. (2000) for a set of commercial hybrids of different breeding eras (range between 70 and 202.7 KNP PGR_{CP}^{-1}). Contrarily, reduced plant reproductive capacity of many inbreds (range between 69 and 138 KNP PGR_{CP}^{-1}) matched the lowest values reported by these authors, which always corresponded to double-cross old hybrids. Additionally, reproductive capacity of some inbreds declined in response to reduced N availability, evidence of further negative effects of this stress on kernel set of this germplasm (D'Andrea et al., 2006) in spite of reduced N requirements for plant growth determined by natural inbreeding depression. This negative effect of N on plant reproductive capacity has been also documented for four out of six commercial maize hybrids in a previous study (D'Andrea et al., 2008a), but was not observed among hybrids used in the present research. Independently of genotypic differences in plant reproductive capacity, a single curvilinear model of the type described by Tollenaar et al. (1992) and Andrade et al. (1999) gave a good fit to most part of the variation in mean values of KNP and PGR_{CP}, with no evident distinction between different genetic materials (e.g., due to heterotic effects) or N levels. Analyses at the individual plant level are necessary for such comparisons (Echarte and Tollenaar, 2006; D'Andrea et al., 2008a).

Reduced plant reproductive capacity of inbreds compared to hybrids could not be attributed to a decreased biomass allocation to ear growth during the critical period (EGR_{CP} PGR_{CP}^{-1}), which was larger for inbreds than for hybrids. Reduced capacity of inbreds was determined by a decline in kernel set per unit EGR_{CP}, represented in the quotient between KN_{E1} and EGR_{CP}. Our findings for a large set of genotypes and growing conditions support results obtained under water-deficit for a reduced set of hybrids (two) and inbreds (three) by Echarte and Tollenaar (2006), and also evidences of increased kernel abortion among inbreds grown under N stress (Monneveux et al., 2005). All these studies, however, did not clarify on biomass distribution within the earshoot (i.e., between vegetative tissue represented by husks plus shank as compare to cob plus florets), which may vary between inbreds and hybrids. Future research should address this issue for a correct assessment of kernel set per unit of biomass allocated in actual ear reproductive tissue.

As mentioned, N supply affected the performance of all genotypes, and the curvilinear response pattern fitted allow to demonstrate that an increase in N absorbed at maturity determined an increase in grain yield of genotypes. Hybrids outyielded inbreds at all levels of N uptake and the response pattern evidenced that N offer at N_0 was very low even for the reduced N requirement of many inbreds. N uptake registered for inbreds (average of 159 and 74 kg N ha^{-1} at N_{400} and N_0, respectively) and hybrids (average of 265 and 75 kg N ha^{-1} at N_{400} and N_0, respectively) in our study was within the range reported for this type of genotypes (between 22 and 185 kg N ha^{-1} for inbreds and between 90 and 270 kg N ha^{-1} for hybrids) which were performed in field experiments under a wide range of environments (Muruli and Paulsen, 1981; Moll et al., 1982; Below et al., 1985; Lafitte and Edmeades, 1994b; Pan et al., 1995; Uhart and Andrade, 1995; Lafitte et al., 1997; Cassman et al., 2003; Coque and Gallais, 2007). Our research, however, is the first to address the variation in response between hybrids and their parental inbreds, which allowed for (i) the determination of potential grain yield of each type of genotype at high N supply (saturation of grain yield represented by the plateau of the curvilinear models in Fig. 4), (ii) the estimation of a similar N uptake threshold for plant barrenness (0.28 g N $plant^{-1}$ for inbreds and 0.34 g N $plant^{-1}$ for hybrids), which has been never reported previously, (iii) the determination of an always improved NUE of hybrids as compared to their parental inbreds, even at very low levels of plant N uptake, and (iv) an apparent enhanced capability to capture N of hybrids at increased N supply. Previous research on this topic (Moll et al., 1982; Bertin and Gallais, 2000; Gallais and Coque, 2005) suggested that differences in grain yield among genotypes were due to (i) differences in NUE at low N rates and (ii) variations in N absorption at high N rates. In our research, at low N levels, grain yield of hybrids was greater (44%) than inbreds although total N uptake did not differ between the two groups. A possible explanation to this is that the higher grain yield was the result of a greater harvest index (23%), which was associated with a greater kernel number (31%), and a greater dry matter accumulation (28%), which was attributable mainly to a higher PAR interception (i.e., RUE was a non-significant 4% greater in hybrids than in inbred lines). The greater KNP (31%) in the hybrids was attributable to a higher PGR_{CP} due to higher PAR interception. This shows that hybrids use the available N more efficiently by, in about equal proportions, (i) a greater PAR interception during the life cycle and (ii) a greater partition of dry matter to the grain. Both effects were the result of the higher leaf area of the hybrids compared to inbreds. Interestingly, hybrids and inbred lines did not differ for mean RUE during the critical period, but RUE of hybrids was greater than that of inbred lines over the whole life cycle. These findings are consistent with results reported by Ahmadzadeh et al. (2004) that showed that leaf photosynthesis did not differ between hybrids and inbred lines until silking, but differences in leaf photosynthesis between the two groups became significant as the genotypes advanced to maturity. The similar RUE observed at low N level may be attributable, in part, to the counteracting effects of (i) a greater reduction in leaf photosynthesis of inbred lines compared to hybrids when the plants advance in development during the grain-filling period (Ahmadzadeh et al., 2004) and (ii) hybrids experienced a greater N stress than inbred lines at the low N level and leaf photosynthesis declines when plant are exposed to N stress (Echarte et al., 2008). In contrast, at high N levels, a large grain yield variation was detected within each genetic material, which may be attributed to differences in N absorption at maturity as well as to improved NUE. In our research, estimated N recovery rate from added fertilizer was low ($\leq$0.48) for both groups of genotypes, probably due to the restriction of having a single and high fertilizer rate for performing this computation. In spite of this constraint, the apparent better capacity to capture N of hybrids than of inbreds at N_{400} may be attributed to reduced growth of inbreds promoted by inbreeding effects. These effects may be linked to (i) a reduced root system of inbreds, with the concomitant reduction in soil exploring capacity or (ii) a reduced N requirement linked to sink limited demand. Future research should address these features in detail.

Finally, the comparisons between different genetic materials revealed that, in general, hybrids were more responsive to variations in N supply than the parental inbreds except for

attributes related to biomass partitioning to reproductive structures (EGR_{CP} PGR_{CP}^{-1} and HI), reproductive capacity (KN_{E1} EGR_{CP}^{-1}), and NUE. This difference between groups was quantified as the relative change in measured traits between N levels, and findings confirmed previous evidence only available for grain yield (Betrán et al., 2003b). Apparently, growth reduction of inbreds promoted by inbreeding depression turned them less susceptible to reduced N availability (e.g., slope of the response curve at threshold of plant N uptake). Contrarily, inbreds were more affected than hybrids in response to others abiotic stress as observed for drought (Betrán et al., 2003b,c) and flooding (Zaidi et al., 2007). This probably indicates that the limited growth promoted by the inbreeding depression determine that a small quantity of N in the soil was enough to an adequate tissue expansion. Oppositely, under water stress, the reduction in water potential below to turgidity level always determined a reduction in the expansion (Ben Haj Salah and Tardieu, 1997; Reymond et al., 2003). These types of stresses represented an additional restriction to already reduced growth.

5. Conclusions

In this research we analyzed maize grain yield determination under contrasting N supply levels for a set of inbred lines and derived single-cross hybrids. Nitrogen stress was different for both types of genotypes, i.e. the low N level was more stressful for the hybrids than for the inbreds. Relevant findings included the detection of genotypic differences in the response to N supply for most measured traits, together with (i) a common pattern across genotypes and N levels for the response of KNP to PGR_{CP}, (ii) a contrasting response of KN_{E1} to EGR_{CP} among different genetic materials, which determined a reduced apical ear reproductive capacity of inbreds as compared to hybrids, (iii) similar RUE during the critical period and N absorption at maturity at low N levels for both groups of genotypes, but enhanced RUE and N absorption of hybrids at high N supply levels, (iv) a strong relationship between N absorption and grain yield but a contrasting response between groups of genotypes, and (v) a reduced NUE of inbreds across all levels of N offer. The improved grain yield of hybrids with similar N absorption at low N levels was attributable to enhanced (i) interception of PAR during the life cycle and (ii) biomass partitioning to the grain. Based on reported evidence, inbreds seems to be inherently more limited than hybrids in their capacity for taking advantage of improved growing conditions (i.e., inbreeding depression of inbreds reduced their tissue expansion capacity and, therefore, their biomass production). In summary, at both N levels, reduced apical ear reproductive capacity was the main physiological limitation to grain yield of inbreds, and reduced NUE was its final consequence.

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Glossary

ASI: anthesis–silking interval
CGR_{CP}: crop growth rate during the critical period
E_1: apical ear
EGR_{CP}: ear growth rate during the critical period
fIPAR: fraction of IPAR intercepted by the canopy
G: genotype
HI: harvest index
KN_{E1}: kernel number per apical ear
KNP: kernel number per plant
KW: kernel weight
LAI: leaf area index
l_n: leaf number n
IPAR: incident PAR
IPARi: daily amount of intercepted IPAR
$IPARi_{CP}$: cumulative IPARi during the critical period
$IPARi_{PM}$: cumulative IPARi at physiological maturity
N: nitrogen
N_n: N level
NUE: N use efficiency
PAR: photosynthetically active radiation
PGR_{CP}: plant growth rate during the critical period
PGY: plant grain yield
PNU_{PM}: plant N uptake at physiological maturity
R_2: onset of active grain filling
RUE: radiation use efficiency
RUE_{CP}: radiation use efficiency during the critical period
TLN: total leaf number
TT: thermal time
V_n: leaf stage n
Y: year