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Parent–Progeny Relationships between Maize Inbreds and Hybrids: Analysis of Grain Yield and Its Determinants for Contrasting Soil Nitrogen Conditions

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ABSTRACT

Most research in maize (*Zea mays* L.) parent–progeny relationships has focused on heterosis for plant grain yield (PGY) determination, whereas nonheterotic effects for traits other than PGY has remained less explored. Our objectives were to analyze (i) frequency distribution and phenotypic plasticity for 29 eco-physiological traits in different genotypic groups (6 inbreds and 12 hybrids) and environments, (ii) parent–progeny relationships for these traits as well as variations in these relationships caused by contrasting growing conditions, and (iii) direct and indirect effects of traits measured in inbreds on hybrid PGY determination. Genotypes were cropped in the field at two contrasting N levels during three growing seasons. Range in phenotypic plasticity was (i) similar for inbreds and hybrids, (ii) largest for traits such as PGY and nitrogen use efficiency (NUE), and (iii) smallest for traits such as time to flowering and kernel weight. Inbred phenotype was usually (26 traits) a good predictor of hybrid phenotype, but analysis of standardized data demonstrated that (i) for nine traits (e.g., PGY, kernel numbers) this relationship was exclusively driven by environmental effects, and (ii) for the other traits there was a true genetic control. A high correlation ($r > 0.26$; $P \leq 0.024$) was established between hybrids PGY and 12 traits measured in inbreds, among which we distinguished NUE and ear growth rate for their high direct effect and participation in the indirect effect of other traits.

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Abbreviations: b_1, regression parameter of mean parent–progeny relationship; b_2, regression parameter of standardized parent–progeny relationship; E, environment; E_1, apical ear; G, genotype; TT, thermal time. Other abbreviations are listed and described in detail in Table 1.

In the main maize (*Zea mays* L.) producing and exporting countries of Europe (France) and the Americas (United States, Brazil, Argentina), commercial production is based on the use of hybrids. For decades, therefore, the challenge for breeders of this species has been the detection of successful combinations of inbreds, aimed at reaching maximum heterosis exploitation (Duvick, 1999; Troyer, 2006) while preserving adequate resistance to diseases and other desirable agronomic traits (e.g., low root and stalk lodging). Therefore, most research effort has focused on understanding differences in grain yield determination between these genotypic groups, with emphasis on the computation of heterosis (i.e., nonadditive genetic effect) in a wide range of environmental conditions (Ahmadzadeh et al., 2004; Tollenaar et al., 2004; Echarte and Tollenaar, 2006; Tollenaar and Lee, 2006; D'Andrea et al., 2009; Munaro et al., 2011a,b). Conversely, progress in our understanding of heritability in parent-progeny relationships (i.e., additive genetic effect) for the physiological and numerical determinants of grain yield has been poor.

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Current state of the art in the interpretation of maize grain yield determination highlights the importance of final kernel number and its response to plant and ear growth rates during the critical period for kernel set (Tollenaar et al., 1992; Andrade et al., 1999; Vega et al., 2001a,b). Relationships among these secondary traits have been thoroughly evaluated for hybrids cropped in potential conditions (Pagano et al., 2007; Pagano and Maddonni, 2007) as well as in abiotic stress environments (Andrade et al., 2002; D'Andrea et al., 2008; Cicchino et al., 2010; Rossini et al., 2012; Rattalino Edreira and Otegui, 2013). The response of inbreds has been much less documented (D'Andrea et al., 2006). Consequently, there is scarce information on parent–progeny relationships. Some concerns one (grain yield) or a few traits (e.g., anthesis-silking interval, leaf chlorophyll, leaf senescence, ears per plant) used in the improvement of maize populations by CIMMYT (Lafitte and Edmeades, 1995; Betrán et al., 2003a; Kebede et al., 2013), but these traits do not fully represent the physiological determinants of grain yield. The most detailed study on inbred–hybrid relationships for these determinants (i.e., resource capture, resource use efficiency for biomass production, and biomass allocation to grains) is restricted to potential growing conditions in the short-season environment of Canada (Tollenaar et al., 2004). This approach limits our understanding of said relationships because (i) the size of the genotype (G) × environment (E) effect differs between hybrids and inbreds, a response that may affect heritability estimates as well as heterosis expression (Munaro et al., 2011b), and (ii) it does not allow for the inclusion of some germplasm of known value for coping with certain abiotic stresses caused by their excessively long cycle for short-season environments (e.g., inbreds derived from tropical populations; Vasal et al., 1999; Betrán et al., 2003b). Therefore, the breadth of the Canadian study is, for the moment, limited to cool temperate environments. Additionally, it gave no information on how abiotic stress conditions may affect mentioned relationships.

An important step in the analysis of genetic effects is the evaluation of trait frequency distribution, including normality analysis. This distribution represents the degree of genotypic variability and is informative of some trait properties. For instance, departure from the statistical ideal of mean zero and normal distribution for bilaterally symmetrical body traits is described as increased fluctuating asymmetry in entomology, a measure of developmental stability that may be indicative of increased homozygosity and/or environmental stress (Palmer and Strobeck, 1992). For a given environment, the extreme situation is represented by platykurtic distributions (e.g., bimodal) that imply marked genetic asymmetry (Palmer and Strobeck, 1992), as may be expected between hybrids and inbreds. Frequency distribution is commonly modified when variation in the environment is included in the analysis, because E and G × E variances sum their effects to the genetic variance (G). This shift in frequency distribution is used for evaluating the degree of trait plasticity (Debat and David, 2001). It is generally accepted that trait heritability decreases when phenotypic plasticity increases because of environmental effects, a condition that penalizes the selection progress as compared with a high-input environment (Bänziger and Lafitte, 1997). In other words, attributes with high parent–progeny relationship (i.e., good predictors) should exhibit low phenotypic plasticity and vice versa (Sadras and Slafer, 2012).

The objectives of the current research were to analyze (i) frequency distribution and phenotypic plasticity for maize grain yield and its physiological and numerical determinants in different genotypic groups (inbreds and hybrids) and environments, (ii) parent–progeny relationships for these traits and variations in these relationships caused by contrasting growing conditions, and (iii) direct and indirect effects of traits measured in inbreds on hybrid grain yield determination. For achieving these objectives, a set of 6 maize inbreds and 12 of their derived hybrids were cropped in the field under contrasting N levels during three growing seasons. Inbreds were of diverse origin (e.g., temperate and Caribbean) and grain type (e.g., dent and flint).

MATERIALS AND METHODS

Genetic Material, 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 to 2003 (Exp. 1), 2003 to 2004 (Exp. 2), and 2004–2005 (Exp. 3). The genetic material evaluated included 12 single-cross maize hybrids (six direct crosses and their reciprocals) selected from all possible crosses of six inbred lines (B100, ZN6, LP662, LP611, LP561, and LP2). Hybrids included in this study were B100 × LP2, B100 × ZN6, B100 × LP561, ZN6 × LP561, ZN6 × LP611, LP561 × LP662, and their reciprocal crosses. Inbreds 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. Inbred B100 is U.S. semi-dent germplasm (Hallauer et al., 1995) and the rest of the inbred lines belong to Argentine flint germplasm. Additionally, inbred LP2 and LP561 were derived from Caribbean germplasm. For details on the method used for hybrid development refer to D'Andrea et al. (2009).

Maize was hand-planted on 1 November, 9 October, and 8 November (Exp. 1 to Exp. 3, respectively). Treatments were a factorial combination of genotypes and two N levels. These levels were a control with no added N (N0, 0 kg N ha^{-1} applied) and a high N condition (N400, 400 kg N ha^{-1}), supplied as urea in two applications between sowing and at the nine-ligulated-leaf stage (V_9; Ritchie et al., 1992). The experimental design was a split plot organized in three randomized complete blocks, with N availability in the main plots and genotypes in the subplots. Each plot had three rows of 5.5-m length with a spacing of 0.7 m

between the rows. Stand density was always 7 plants m^{-2}. Supplemental irrigation was given to prevent water stress by keeping the uppermost 1 m of soil near field capacity. The topsoil (0–40-cm layer) had an organic matter content of 22 (Exp. 1 and Exp. 2) and 14 g kg^{-1} (Exp. 3). No P fertilizer was needed. Inorganic N at sowing was 55, 23, and 40 g kg^{-1} (Exp. 1 to Exp. 3, respectively). 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) 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 °Cd with base temperature of 8°C) was computed from mean daily air temperatures from sowing onward as proposed by Ritchie and NeSmith (1991).

Measurements

Twenty-nine traits comprising the main physiological and numerical determinants of grain yield and N metabolism were measured (Table 1). Methods for the assessment of these traits were well described in D'Andrea et al. (2009) and Munaro et al. (2011a). Briefly, five successive plants were tagged at V_3 on the central row of each plot, and most traits of interest were estimated from these plants. Individual leaf area was computed as lamina length × maximum width × 0.75 (Montgomery, 1911), and plant leaf area was obtained by summing all green leaves measured on each plant around flowering and at maturity. Maximum leaf area index (LAI_{MAX}) and leaf area index at physiological maturity (LAI_{PM}) were 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]

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

where I_b is the incident photosynthetically photon flux density immediately below the bottommost green leaves and I_a is the incident photosynthetically photon flux density at the top of the canopy (Gallo and Daughtry, 1986). Measurements were made with a line quantum-sensor (Cavabar, Cavadevices) at a rate of three determinations per plot for I_b and one for each of five plots for I_a. Daily fIPAR values were obtained from nonlinear models (e.g., Gaussian or logistic functions) fitted to observed data. The selection of the model in each genotype × N level × replication was based on the r^2 value. The parameters of the selected models were fitted using the iterative optimization technique of Table Curve 3.0 (Jandel, 1992). The daily amount of intercepted IPAR (IPARI) was computed as the product between fIPAR and IPAR, and cumulated values were obtained for (i) the critical period for kernel set between approximately V_{14} and R_2 (i.e., $IPARI_{CP}$) and (ii) the grain-filling period between R_2 and physiological maturity (i.e., $IPARI_{GF}$). $IPARI_{CP}$ was expressed on a daily basis for comparison with other traits computed for the same period.

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 (TT_{ANT}) and silking (TT_{SILK}) were determined for each entry.

Biomass production was estimated at V_{14} and at R_2 ($BIOM_{SILK}$) by means of allometric models (Vega et al., 2000; Borrás and Otegui, 2001; Maddonni and Otegui, 2004). This approach 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; Cicchino et al., 2010). 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 grams per day) and ear growth rate (EGR_{CP}; in grams per day) 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 (i) the critical period for kernel set between approximately V_{14} and R_2 (i.e., RUE_{CP}) and (ii) for the grain-filling period between R_2 and physiological maturity (i.e., RUE_{GF}). All tagged plants were individually harvested at physiological maturity. Plant material was oven-dried at 60°C for 7 d and weighed for final shoot plant biomass determination ($BIOM_{PM}$). 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 (KNE_1) and subapical ear (when present). Grain yield was computed for each harvested plant (PGY), and individual kernel weight (KW) 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 $BIOM_{PM}$; (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}.

Nitrogen concentration was determined for vegetative tissues (leaves, stem, husks, and cob) and grains of each plant harvested at maturity. Micro Kjeldahl analysis was used for the vegetative fraction, and near infrared transmittance (Infratec 1227, Tecator) for the grain fraction (%Protein). Nitrogen content (in grams per plant) 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 (PN_{UPTAKE}). Nitrogen utilization efficiency (NUE) was computed as the quotient between PGY and PN_{UPTAKE}, N harvest index (NHI) as the quotient between grain N and $BIOM_{PM}$, and N proportion as the ratio between PN_{UPTAKE} and $BIOM_{PM}$ ($N/BIOM_{PM}$).

STATISTICAL ANALYSIS

Each attribute described in this work (Table 1) was evaluated by an ANOVA test performed across experiments, N levels, genotypes, and all possible interactions by means of PROC GLM procedure of SAS v. 8.2 (SAS Institute, 1999). Mean values and significance levels were described in detail in D'Andrea et al. (2009). Briefly, we detected a significant genotypic effect ($P < 0.01$) for all measured traits, whereas N effect was significant ($P < 0.10$) for most traits except ASI, $IPARI_{CP}$, EGR_{CP}/PGR_{CP}, HI, and KNE_1/EGR_{CP}. The G × N interaction was significant

Table 1. Abbreviation and description of evaluated traits.

Group	Abbreviation	Trait description
Phenology	TT_{ANT}	Thermal time to anthesis (°Cd)
	TT_{SILK}	Thermal time to silking (°Cd)
	ASI	Anthesis–silking interval (days)
Tissue expansion and light capture	LAI_{MAX}	Maximum leaf area index (green leaf m^2 soil m^{-2})
	LAI_{PM}	Green leaf area index at physiological maturity (green leaf m^2 soil m^{-2})
	$fIPAR_{MAX}$	Maximum proportion of incident PAR intercepted
	$fIPAR_{PM}$	Proportion of incident PAR intercepted at physiological maturity
	$IPARI_{CP}$	Amount of incident PAR intercepted daily during the critical period (MJ m^{-2} d^{-1})
	$IPARI_{GF}$	Amount of incident PAR intercepted during grain filling (MJ m^{-2})
	$IPARI_{PM}$	Amount of incident PAR intercepted up to physiological maturity (MJ m^{-2})
Photosynthetic capacity and biomass production	PGR_{CP}	Plant growth rate during the critical period (g d^{-1})
	$BIOM_{SILK}$	Plant biomass at silking (g)
	$BIOM_{PM}$	Plant biomass at physiological maturity (g)
	RUE_{CP}	Radiation use efficiency during the critical period (g MJ^{-1})
	RUE_{GF}	Radiation use efficiency during grain filling (g MJ^{-1})
	RUE_{PM}	Radiation use efficiency to physiological maturity (g MJ^{-1})
Biomass partitioning and reproductive efficiency	EGR_{CP}	Ear growth rate during the critical period (g d^{-1})
	EGR_{CP}/PGR_{CP}	Biomass partitioning to the ear during the critical period
	KNP/PGR_{CP}	Plant biomass reproductive efficiency (kernels d g^{-1})
	KNE_1/EGR_{CP}	Apical ear biomass reproductive efficiency (kernels d g^{-1})
	HI	Harvest index (PGY $BIOM_{PM}^{-1}$)
Grain yield and its components	KNP	Kernel number per plant
	KW	Individual kernel weight (mg)
	PGY	Plant grain yield (g)
N metabolism	PN_{UPTAKE}	Total N uptake per plant at physiological maturity (g)
	%Protein	Percent grain protein
	NHI	N harvest index (grain N $BIOM_{PM}$ N^{-1})
	$N/BIOM_{PM}$	N proportion in plant biomass at physiological maturity
	NUE	N use efficiency for grain production (g grain g N^{-1})

($P < 0.05$) for all traits except TT, ASI, $fIPAR_{MAX}$, and KNE_1/EGR_{CP}. Mean values of inbreds and hybrids differed ($P < 0.05$) across evaluated environments (i.e., year × N level) for all traits except RUE_{CP}. Hybrid PGY was reduced 40% by N deficiency (i.e., from 862 g m^{-2} at N400 to 514 g m^{-2} at N0), whereas inbred PGY was reduced only 24% (i.e., from 382 g m^{-2} at N400 to 288 g m^{-2} at N0). Similar patterns were observed in most traits, that is, the negative effects of N deficiency were always larger among hybrids (e.g., decrease of 72% for PN_{UPTAKE}, 29% for KNP, 31% for $BIOM_{PM}$, and 61% for LAI_{PM}) than among inbreds (e.g., decrease of 53% for PN_{UPTAKE}, 20% for KNP, 17% for $BIOM_{PM}$, and 31% for LAI_{PM}).

Frequency distributions were computed for each trait to evaluate the range and type of variation produced by the selected genotypes. A separate analysis was made for each genotypic group × N level combination, to eliminate N and heterosis effects. Each data set consisted of 18 observations for inbred (6 genotypes × 3 experimental years) and 36 observations for hybrids (12 genotypes × 3 experimental years) in each N level. Subsequently, traits were normalized for a common comparison of phenotypic plasticity (Sadras and Slafer, 2012). For this purpose, the 50th (median), 10th, and 90th percentiles were obtained for each specific genotype (12 hybrids and 6 inbreds) and then averaged within each genotype group. Median value was set to 1, and the 10th and 90th percentiles were expressed as ratios with the median of each attribute.

Parent–progeny relationships (Falconer, 1989) were calculated for each trait as the parameter b of the regression between (i) mean parental (inbred) and progeny (hybrid) values (b_1), and (ii) standardized (i.e., each value was subtracted from the mean of genotypic group × N level × Year combination and was divided by the standard deviation) parental and progeny values (b_2). For regression of mean values, an F test was used for comparison of slopes between models fitted to each N level (Balzarini and Di Rienzo, 2012). Standardized values eliminated the bias caused by heterosis (evident as a departure from the 1:1 relationship and/or $b_1 > 1$) and N effects (evident as b_1 N0 ≠ b_1 N400). Therefore, parameter b_2 gave an enhanced estimation of additive effects on the phenotypic variance.

Path analysis (Dewey and Lu, 1959; Board et al., 1997) was applied to the standardized data set (Balzarini and Di Rienzo, 2012) to determine the direct and indirect contribution (path coefficients) of predictor variables (in this case inbred traits) on a response variable (in this case hybrid PGY). Correlation coefficients (r) between each trait surveyed in inbred and hybrid PGY correspond to the sum of the direct plus all indirect effects of an independent variable X_1 on a dependent variable Y (Cramer et al., 1999). Indirect effects of X_1 are those mediated by other variables (X_2.... X_n), and the path coefficient of each indirect effect of X_1 corresponds to the product between the direct effect of the other variable (e.g., X_2) on Y and the correlation between independent variables (in this case X_1 and X_2).

RESULTS

Trait Frequency Distribution and Phenotypic Plasticity

Genotypes included in this study covered a wide range of values for all evaluated traits (Table 2), both within inbreds and hybrids. This was true even for the high-input environment represented by irrigated and fertilized plots (i.e., N400). In spite of this trend, results obtained in this condition detected no departure from normality for the limited set of parental inbreds used in this work (Table 2). Moreover, cases of non-normal distribution were scarce (maximum of 6 out of 29 traits for hybrids at N400) and had no clear trend (i.e., these traits were not the same for a given genotypic group across N levels, except for RUE_{PM} and KNE_1/EGR_{CP} in hybrids).

Table 2. Descriptive statistics of measured traits for each genotype group × N combination.

	N400						N0					
	Inbreds			Hybrids			Inbreds			Hybrids		
Trait†	Range‡	Median	S–W§	Range	Median	S–W	Range	Median	S–W	Range	Median	S–W
TT_{ANT}	919–1118	1009	ns	828–988	899	ns	913–1111	1016	ns	846–1006	921	ns
TT_{SILK}	913–1171	1025	ns	820–1030	912	ns	908–1199	1057	ns	847–1066	956	ns
ASI	–0.61–5.87	2.99	ns	–1.27–5.19	1.3	ns	–0.41–9.28	3033	ns	0.07–5.75	2.98	ns
LAI_{MAX}	2.24–4.23	3.04	ns	3.84–6.30	4.56	1.32/1.38	2.11–4.17	2.52	1.36/1.32	2.78–4.19	3.31	ns
LAI_{PM}	0.00–3.91	1.91	ns	2.04–5.84	3.93	ns	0.24–3.22	1.11	ns	0.55–2.39	1.46	ns
$fIPAR_{MAX}$	0.59–0.86	0.75	ns	0.85–0.96	0.91	ns	0.43–0.87	0.59	ns	0.61–0.88	0.81	–0.77/–0.60
$fIPAR_{PM}$	0.02–0.72	0.5	ns	0.49–0.91	0.78	ns	0.08–0.68	0.30	ns	0.25–0.65	0.45	ns
$IPARI_{CP}$	6.22–8.70	7.69	ns	8.70–11.21	9.76	ns	4.85–8.47	6.52	ns	6.05–10.14	8.77	–0.45/–1.16
$IPARI_{GF}$	78–339	227	ns	243–501	398	ns	72–250	160	ns	161–295	240	ns
$IPARI_{PM}$	408–702	545	ns	689–958	814	ns	318–655	463	ns	476–746	631	ns
PGR_{CP}	1.80–3.47	2.41	ns	3.02–5.05	4.04	ns	1.48–2.90	2.23	ns	1.88–3.19	2.66	ns
$BIOM_{SILK}$	71–167	114	ns	132–232	169	ns	65–148	95	ns	100–155	126	ns
$BIOM_{PM}$	113–222	165	ns	247–345	299	ns	96–177	136	ns	144–231	189	ns
RUE_{CP}	1.76–3.25	2.5	ns	2.53–3.73	3.04	ns	1.68–3.24	2.55	ns	1.97–3.00	2.39	ns
RUE_{GF}	0.95–2.54	1.64	ns	1.53–3.39	2.29	ns	0.59–2.39	1.54	ns	0.65–2.86	1.8	ns
RUE_{PM}	1.78–2.77	2.33	ns	2.45–3.75	2.75	1.16/1.74	1.43–2.94	2.27	ns	1.71–2.98	2.2	0.47/–0.92
EGR_{CP}	0.41–1.66	0.91	ns	0.55–1.53	1.13	–0.66/–0.08	0.43–1.27	0.68	ns	0.46–1.13	0.74	ns
EGR_{CP}/PGR_{CP}	0.18–0.49	0.36	ns	0.16–0.39	0.28	ns	0.19–0.45	0.34	ns	0.15–0.38	0.29	ns
KNP/PGR_{CP}	61–156	108	ns	83–144	118	ns	48–157	105	ns	93–147	124	ns
KNE_1/EGR_{CP}	216–490	313	ns	309–600	431	0.63/–0.35	168–417	325	ns	340–633	446	0.51/–0.80
HI	0.16–0.42	0.34	ns	0.28–0.49	0.42	–0.77/0.01	0.12–0.43	0.31	ns	0.32–0.46	0.38	ns
KNP	132–388	275	ns	308–540	447	ns	101–356	220	ns	223–414	312	ns
KW	158–232	198	ns	244–309	273	ns	150–227	189	ns	205–256	229	ns
PGY	25–81	57	ns	77–158	125	ns	17–60	41	ns	48–101	71	ns
PN_{UPTAKE}	1.62–2.94	2.16	ns	2.92–4.55	3.73	ns	0.71–2.24	1.13	1.48/2.36	0.88–1.82	1.09	0.94/–0.16
%Protein	10.7–14.0	12.4	ns	9.5–12.1	10.7	ns	6.9–12.9	9.6	ns	5.1–9.3	6.7	ns
NHI	0.23–0.63	0.5	ns	0.40–0.65	0.58	–1.06/0.84	0.25–0.68	0.57	–0.97/–0.18	0.55–0.73	0.65	ns
$N/BIOM_{PM}$	0.012–0.017	0.013	ns	0.011–0.014	0.012	ns	0.007–0.013	0.008	1.65/2.04	0.005–0.008	0.0065	ns
NUE	10.9–35.2	25.2	ns	20.7–43.0	34.7	ns	12.2–52.7	38	ns	40.3–80.4	62.6	ns

†Description in Table 1.

‡Minimum and maximum values. For inbreds $n = 18$ (6 genotypes × 3 experimental years) and for hybrids $n = 36$ (12 genotypes × 3 experimental years).

§Shapiro–Wilk normality test: ns indicates not significantly different from normal ($P > 0.05$); values represent skew and kurtosis coefficients when $P \leq 0.05$.

For a given N level, differences were observed in frequency distribution curves between inbreds and hybrids for most traits (Fig. 1). Ranges explored were (i) always contrasting for traits, as KNP (Fig. 1a), PGY, KW, biomass production, PGR_{CP}, LAI, IPARI, and HI (i.e., large separation between inbreds and hybrids in their frequency distribution curves); (ii) similar for traits such as KNP/PGR_{CP} (Fig. 1b), TT_{ANT}, TT_{SILK}, EGR_{CP}/PGR_{CP}, ASI, and RUE_{CP} (i.e., overlapping in previously mentioned curves); and (iii) contrasting only at high N level for traits, as EGR_{CP}, $fIPAR_{PM}$, RUE_{GF}, and RUE_{PM}. Regarding N effects, ranges explored by frequency distribution curves were (i) similar for traits such as EGR_{CP}/PGR_{CP} (Fig. 1c), KNP/PGR_{CP}, and KNE_1/EGR_{CP} (i.e., overlapping of N0 and N400 curves within each genotypic group); (ii) always higher at N400 for traits such as $N/BIOM_{PM}$ (Fig. 1d), %Protein, PN_{UPTAKE}, $fIPAR_{MAX}$, and $IPARI_{CP}$, (iii) always higher at N0 for traits, as NUE (Fig. 1e) and NHI; or (iv) contrasting on one group of genotypes (hybrids) but similar on the other (inbreds), as for RUE_{CP} (Fig. 1f), LAI_{MAX}, LAI_{PM}, $fIPAR_{PM}$, $IPARI_{GF}$, $IPARI_{PM}$, all biomass production and RUE traits, PGY, KNP, and KW.

Considering the whole data set (i.e., across genotypes, N, and years), phenotypic plasticity was largest for ASI, LAI_{PM}, $fIPAR_{PM}$, PGY, PN_{UPTAKE}, and NUE (Fig. 2); that is, variation with respect to the median value was >50% at least at one tail of the distribution of one genotypic group. On the contrary, traits such as TT_{ANT}, TT_{SILK}, and KW had the smallest plasticity; that is, variation with respect to the median value was always <25% at both tails of the distribution. All other traits had an intermediate phenotypic plasticity; in other words, variation with respect to the median value was always between 25 and 50% at any tail of the distribution.

In general, the 10th percentile represented the poor environment (N0) and the 90th percentile represented the rich environment (N400), as observed for PGY, KW, and PN_{UPTAKE}. This trend was opposite for NUE, ASI, TT_{ANT}, and TT_{SILK}. For the first group of traits, the increase of the 90th percentile with respect to the median value could be attributed predominantly to improved soil N and reached

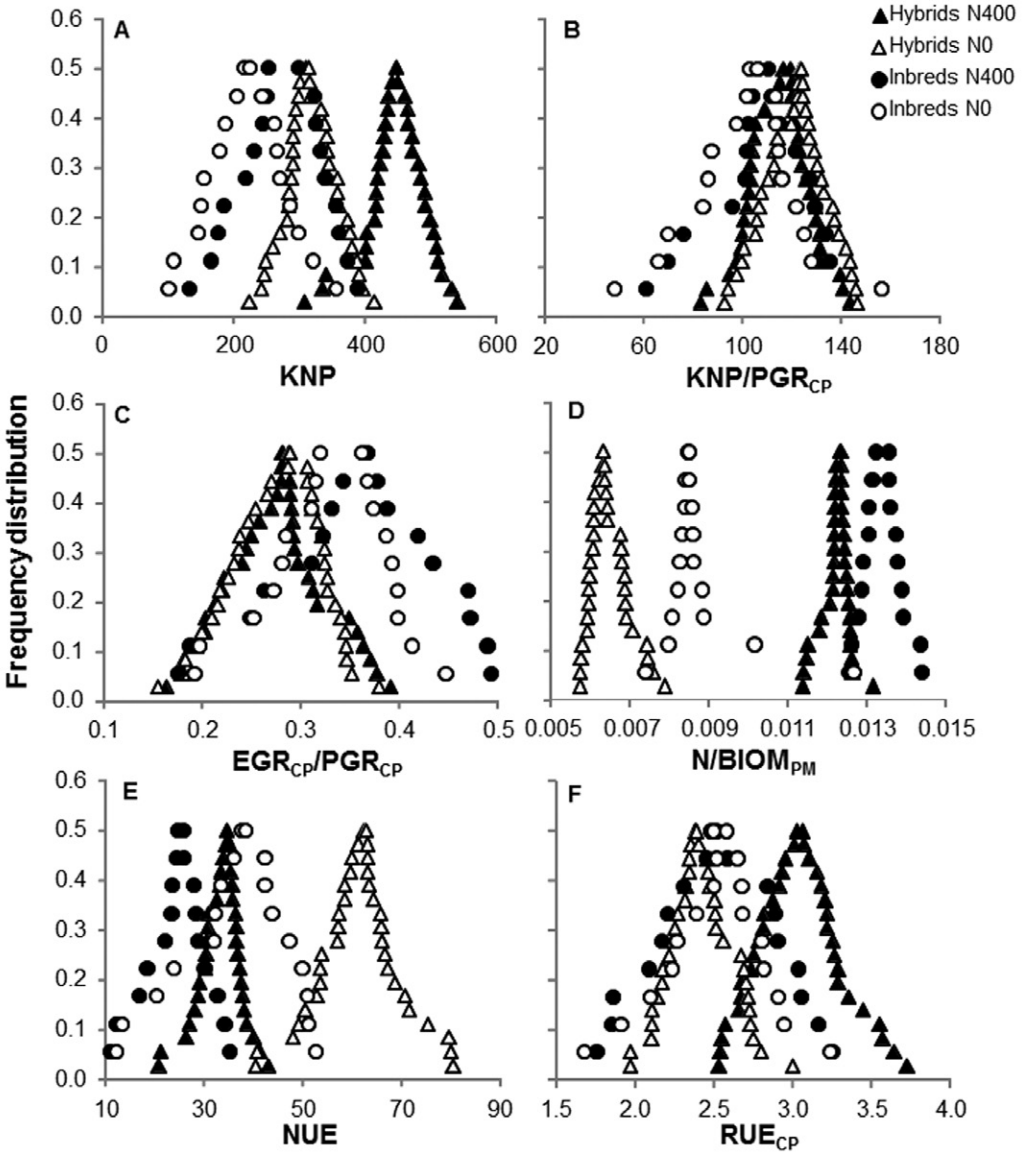

Figure 1. Frequency distribution of (A) kernel number per plant (KNP), (B) KNP per unit plant growth rate during the critical period (KNP/PGR$_{CP}$), (C) ear growth rate during the critical period (EGR$_{CP}$) per unit PGR$_{CP}$, (D) N per unit biomass at physiological maturity (N/BIOM$_{PM}$), (E) N use efficiency (NUE), and (F) radiation use efficiency during the critical period (RUE$_{CP}$). Data correspond to 6 inbred lines and their 12 derived hybrids cropped at two N levels (N0 and N400) during three experimental years (i.e., n = 18 for inbreds and n = 36 for hybrids in each N level).

65% for LAI$_{PM}$, 60% for PN$_{UPTAKE}$, 50% for PGY, 45% for IPARI$_{GF}$, 30% for BIOM$_{PM}$, and only 15% for HI and KW (Fig. 2). For these traits, reduced N availability at N0 caused a decrease with respect to the median value of 65% for LAI$_{PM}$, 55% for PN$_{UPTAKE}$, 40% for PGY, 30% for IPARI$_{GF}$ and BIOM$_{PM}$, and only 20 and 15% for HI and KW, respectively. This trend was represented by the 10th percentile (Fig. 2). As mentioned above, the response to soil N availability was exactly opposite for the second group of traits, which decreased at N400 (70% for ASI and 35% for NUE, represented by the 10th percentile) and increased at N0 (75% for ASI and 60% for NUE, represented by the 90th percentile).

In spite of the usually contrasting range explored by inbreds and hybrids (Table 2; Fig. 1), both groups of genotypes had a similar phenotypic plasticity (Fig. 2) in attributes KW, LAI$_{MAX}$, RUE$_{CP}$, BIOM$_{SILK}$, IPARI$_{CP}$, and TT. This was independent of the plasticity level (e.g., intermediate for BIOM$_{SILK}$ and low for TT). For other attributes, however, the magnitude of phenotypic plasticity differed between inbreds and hybrids. For instance, plasticity in HI and NHI was always (i.e., at both N levels) larger for inbreds than for hybrids, and the opposite was true for PN$_{UPTAKE}$ (Fig. 2). For a third group of attributes, differences in plasticity between inbreds and hybrids were conditioned by N level. For instance, phenotypic plasticity at N400 was larger for inbreds than for hybrids in ASI (in this case represented by the 10th percentile), KNP, fIPAR$_{PM}$, and RUE$_{GF}$ (in these cases represented by the 90th percentile).

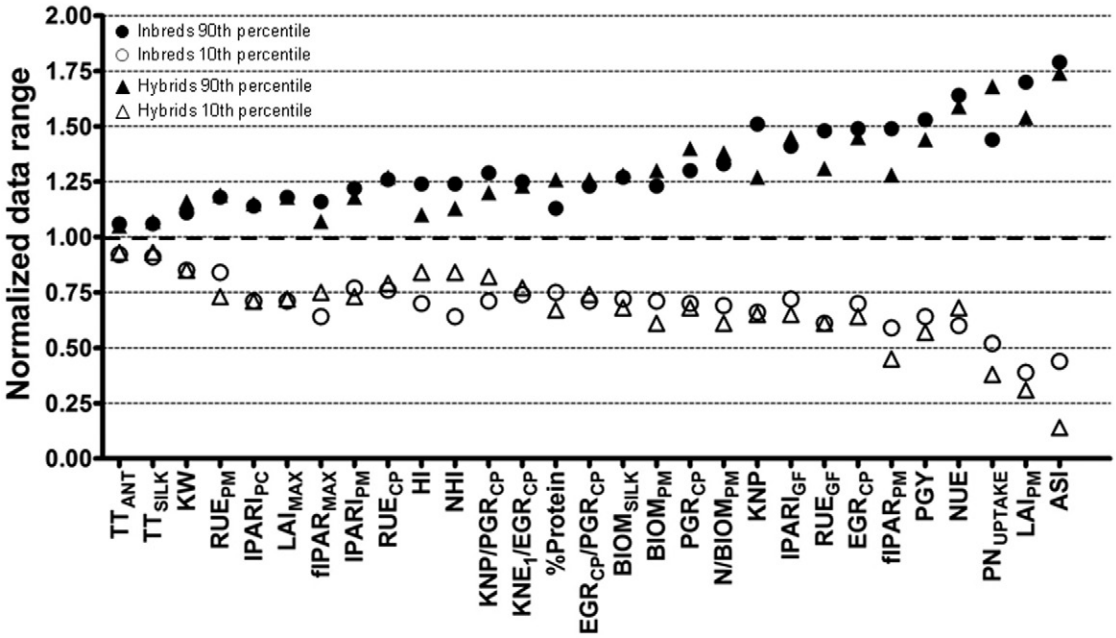

Figure 2. Phenotypic plasticity of 29 attributes (listed in Table 1) measured at two N levels (N0 and N400) during three experimental years in 6 inbred lines and their 12 derived hybrids. The median (dashed line) is set to 1, and the 10th and 90th percentiles are expressed as ratios with respect to the median value.

Parent–Progeny Relationships

We distinguished three groups of attributes based on parent–progeny relationships fitted to mean data values (Table 3): one group (Group 1) for which inbred performance gave a good prediction of hybrids performance at both N levels ($P \leq 0.05$), a second group (Group 2) for which no inference could be made from the parent–progeny relationship at any N level ($P > 0.05$), and a third group (Group 3) for which the relationship held only at one N level (N0 or N400). Phenology traits (TT_{ANT}, TT_{SILK}, and ASI) as well as LAI_{MAX}, $fIPAR_{MAX}$, $IPARI_{CP}$, $IPARI_{GF}$, $IPARI_{PM}$ (Fig. 3a), PGR_{CP}, $BIOM_{SILK}$, $BIOM_{PM}$, RUE_{GF}, RUE_{PM}, EGR_{CP}/PGR_{CP}, KNE_1/EGR_{CP}, HI, KW, PN_{UPTAKE}, and NUE corresponded to the first group (Table 3). Within this group, fitted models did not differ between N levels (Fig. 3a), except for $fIPAR_{MAX}$, $IPARI_{CP}$, $IPARI_{GF}$, and HI (Fig. 3c). Traits such as RUE_{CP} (Fig. 3e) and KNP/PGR_{CP} belonged to the second group, and all other traits belonged to the third group (Table 3). For the latter, the parent–progeny relationship held (i) only at N400 for LAI_{PM}, $fIPAR_{PM}$, EGR_{CP}, KNP, PGY (Fig. 3 g), and NHI; and (ii) only at N0 for %Protein and $N/BIOM_{PM}$.

Heterotic effects produced a positive departure from the 1:1 relationship (Fig. 3a, c, e, g) and/or $b_1 > 1$ (Table 3). However, trends in parent–progeny relationships (i.e., groups described above) had no clear association with heterosis, which was low or null for phenology traits (Group 1) as well as for RUE_{CP} and KNP/PGR_{CP} (Group 2) and always very high for traits related to light capture and biomass production (most in Group 1). Normalization eliminated (i) heterotic effects, evident as no clustering of data on one side of the 1:1 relationship (Fig. 3b, d, f) and $b_2 < 1$ in all cases with significant ($P \leq 0.05$) parent–progeny relationship (Table 3; Fig. 3b, d, f); and (ii) trends caused by N (Fig. 3d) and/or year effects. After this procedure a single model gave a good fit to the whole data set of traits for which a significant ($P \leq 0.05$) parent–progeny relationship could still be detected. Most evaluated traits (20 out of 29) fulfilled this condition, except $IPARI_{CP}$, PGR_{CP}, $BIOM_{SILK}$, RUE_{GF}, RUE_{PM}, KNE_1/EGR_{CP}, KNP, PGY (Fig. 3h), and $N/BIOM_{PM}$ (Table 3). For the latter, significant relationships ($P \leq 0.05$) fitted to mean inbred and hybrid values were mostly driven by heterotic and/or environmental (i.e., N and year) effects and not by additive genotypic effects.

Path Analysis for Trait Hierarchy Definition

On the basis of standardized values, only 12 of 29 traits evaluated in inbreds had a significant ($P \leq 0.05$) correlation with PGY of hybrids (Table 4). Among these traits, some had a positive and others a negative influence on the dependent variable. Enhanced ear growth and biomass allocation to reproductive organs (EGR_{CP}, EGR_{CP}/PGR_{CP}, and HI) as well as increased use of absorbed N for grain production (NUE, NHI) belonged to the first group. Extended time to silking (TT_{SILK}) and asynchrony between pollen shed and silks receptivity (ASI), as well as enhanced kernel set per unit of apical ear growth (KNE_1/EGR_{CP}) and leaf area duration (LAI_{PM}) and its effects on light interception ($fIPAR_{PM}$, $IPARI_{GF}$, $IPARI_{PM}$), belonged to the second group. In some cases their influence derived from predominant direct effects, as for LAI_{PM}, EGR_{CP}, and NUE. For these traits the path

Table 3. Parent–progeny relationships.

Group	Trait†	Parent–progeny relationship of mean values: N400 b_1‡	N400 r^2§	N400 P	N0 b_1	N0 r^2	N0 P	Comparison of slopes¶	Parent–progeny relationship of standardized values b_2	r^2#	P
Phenology	TT_{ANT} (°Cd)	0.65	0.71	<0.001	0.73	0.69	<0.001	ns	0.822	0.675	<0.001
	TT_{SILK} (°Cd)	0.73	0.70	<0.001	0.84	0.74	<0.001	ns	0.846	0.716	<0.001
	ASI (days)	0.92	0.52	<0.001	0.84	0.75	<0.001	ns	0.851	0.724	<0.001
Tissue expansion and light capture	LAI_{MAX}	1.13	0.45	<0.001	0.62	0.28	0.001	ns	0.552	0.304	<0.001
	LAI_{PM}	1.05	0.51	<0.001	0.07	0.00	ns	–	0.424	0.180	<0.001
	$fIPAR_{MAX}$	0.21	0.30	0.001	0.64	0.57	<0.001	0.0015	0.382	0.146	0.001
	$fIPAR_{PM}$	0.34	0.38	<0.001	0.12	0.01	ns	–	0.466	0.217	<0.001
	$IPARI_{CP}$ (MJ d^{-1} m^{-2})	0.76	0.18	0.01	1.52	0.73	<0.001	0.0145	0.195	0.038	ns
	$IPARI_{GF}$ (MJ m^{-2})	1.28	0.73	<0.001	0.63	0.17	0.013	0.0295	0.642	0.412	<0.001
	$IPARI_{PM}$ (MJ m^{-2})	0.93	0.46	<0.001	1.15	0.73	<0.001	ns	0.636	0.404	<0.001
Photosynthetic capacity and biomass production	PGR_{CP} (g d^{-1})	0.48	0.13	0.032	0.56	0.41	<0.001	ns	–0.014	<0.001	ns
	$BIOM_{SILK}$ (g)	0.66	0.33	<0.001	0.50	0.33	<0.001	ns	–0.006	<0.001	ns
	$BIOM_{PM}$ (g)	0.78	0.41	<0.001	0.57	0.25	0.002	ns	0.294	0.086	0.012
	RUE_{CP} (g MJ^{-1})	0.08	0.01	ns	0.15	0.03	ns	–	0.292	0.082	0.015
	RUE_{GF} (g MJ^{-1})	0.67	0.20	0.007	1.09	0.39	<0.001	ns	0.045	0.002	ns
	RUE_{PM} (g MJ^{-1})	0.95	0.38	<0.001	0.83	0.24	0.002	ns	0.207	0.043	ns
Biomass partitioning and reproductive efficiency	EGR_{CP} (g d^{-1})	0.59	0.30	0.001	0.21	0.00	ns	–	0.521	0.272	<0.001
	EGR_{CP}/PGR_{CP}	0.61	0.53	<0.001	0.72	0.42	<0.001	ns	0.561	0.314	<0.001
	KNP/PGR_{CP} (kernels d g^{-1})	0.10	0.01	ns	0.09	0.01	ns	–	0.278	0.077	0.02
	KNE_1/EGR_{CP} (kernels d g^{-1})	0.92	0.35	<0.001	0.82	0.16	0.014	ns	0.204	0.041	ns
	HI	0.66	0.55	<0.001	0.24	0.16	0.015	0.0033	0.684	0.468	<0.001
Grain yield and its components	KNP	0.48	0.40	<0.001	0.22	0.07	ns	–	0.117	0.014	ns
	KW (mg)	1.00	0.32	<0.001	0.50	0.16	0.014	ns	0.537	0.288	<0.001
	PGY (g)	0.88	0.51	<0.001	0.16	0.02	ns	–	0.201	0.040	ns
N metabolism	PN_{UPTAKE} (g)	0.59	0.19	0.008	0.66	0.65	<0.001	ns	0.497	0.247	<0.001
	%Protein	0.06	0.00	ns	0.38	0.58	<0.001	–	0.250	0.062	0.03
	NHI	0.38	0.43	<0.001	0.06	0.02	ns	–	0.272	0.062	0.03
	$N/BIOM_{PM}$	–0.05	0.01	ns	0.33	0.39	<0.001	–	0.031	0.001	ns
	NUE	0.54	0.43	<0.001	0.69	0.48	<0.001	ns	0.592	0.351	<0.001

†Description in Table 1.

‡b_n coefficient of parent–progeny regression, for each N level (N400 and N0) across experiments (b_1) or across N levels and experiments (b_2).

§Regression with n = 36 (12 genotypes × 3 experimental years) for each N level.

¶b_1 N400 vs. b_1 N0, ns = not significant.

#Regression with n = 72 (12 genotypes × 2 N levels × 3 experimental years).

coefficient of direct effects was large (absolute value ≥ 1) and of the same sign as the mentioned correlation coefficient (e.g., negative for LAI_{PM} and positive for EGR_{CP} and NUE). For the other traits the direct effect was (i) small, as for TT_{SILK}, ASI, $IPARI_{GF}$, $IPARI_{PM}$, KNE_1/EGR_{CP}, and NHI (absolute value of the path coefficient < 1); or (ii) large but had a sign opposite to the correlation coefficient, as for $fIPAR_{PM}$, EGR/PGR_{CP}, and HI. For these other traits, the trend of the correlation with hybrid PGY was predominantly conditioned by indirect effects (i.e., mediated by other independent variable/s that may or may not have a significant correlation with hybrid PGY).

A conceptual summary of the most important direct and indirect effects of traits measured in parental inbreds on hybrid PGY is given in Fig. 4, together with general trends in the correlations between traits measured in inbreds (Pearson's correlation matrix not shown). For instance, the positive correlation of inbred HI with hybrid PGY (r = 0.40, P < 0.001; Table 4) could not be attributed to the direct effect of the former on the latter (evident as a negative path coefficient of -3.12; Table 4 and Fig. 4) but to indirect effects of HI mediated by its (i) negative correlation with LAI_{PM} (r = -0.74) and (ii) positive correlation with PGY (r = 0.60) and NUE (r = 0.96). For the former, the negative correlation between HI and LAI_{PM} together with the large negative direct effect of LAI_{PM} on hybrid PGY (-2.21; Table 4 and Fig. 4) produced a final positive indirect effect of HI (-0.74 × -2.21 = 1.64; Table 4) on the response variable. The opposite can be deduced for the positive indirect effect of HI through NUE (2.91; Table 4), which derived from the positive correlation between these traits combined with the large positive direct effect of NUE on hybrid PGY (3.04; Table 4 and Fig. 4). Similar comments deserve the trends registered for other traits with a direct effect opposite in sign to their correlation with hybrids PGY, independently of the magnitude of that effect (e.g., large for EGR_{CP}/PGR_{CP} and $fIPAR_{PM}$ or small for NHI).

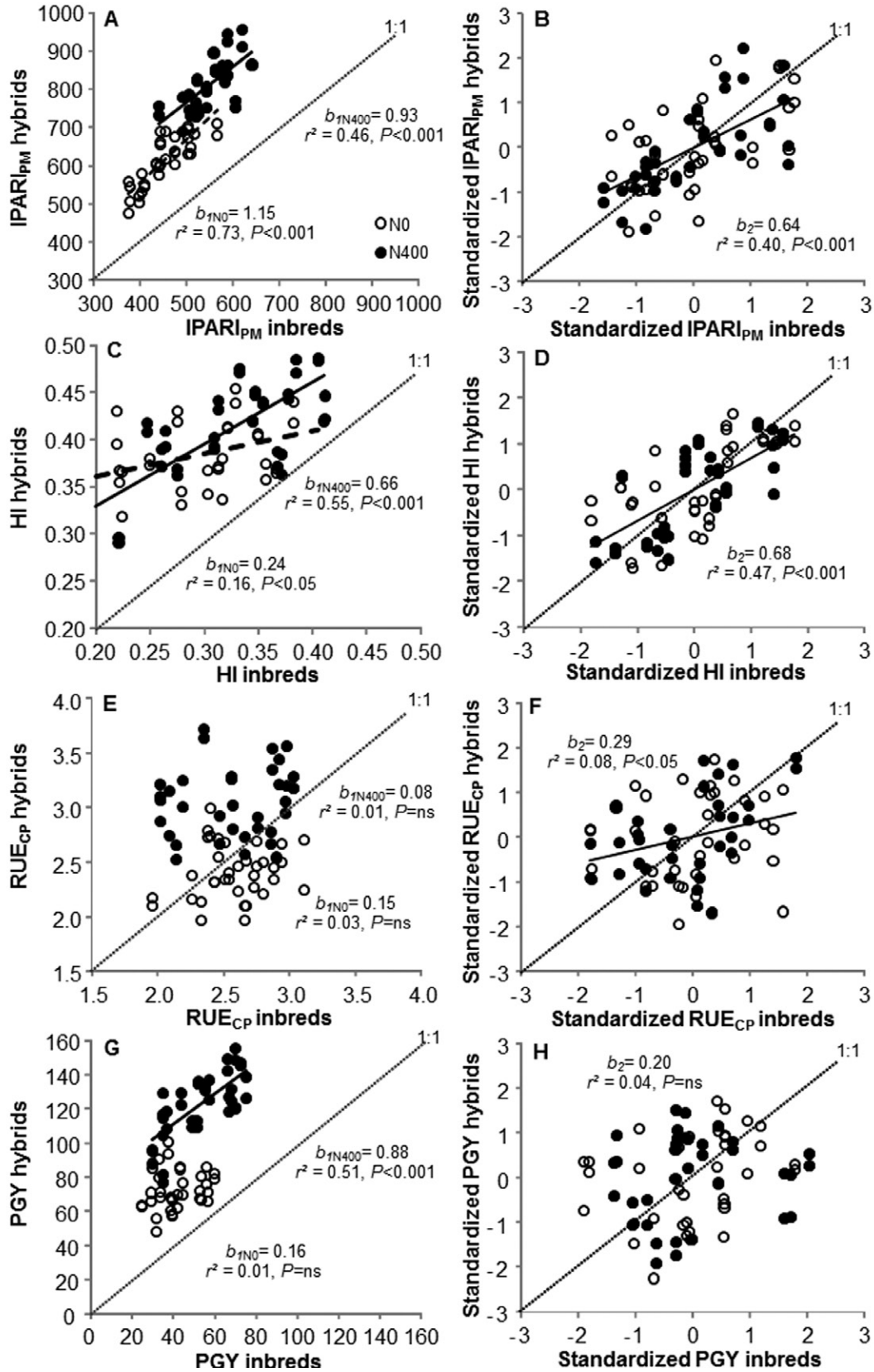

Figure 3. Parent–progeny relationships of mean values (A, C, E, G) and standardized parent–progeny relationships (B, D, F, H) of (A and B) the amount of incident photosynthetically active radiation intercepted up to physiological maturity ($IPARI_{PM}$), (C and D) harvest index (HI), (E and F) radiation use efficiency during the critical period (RUE_{CP}), and (G and H) plant grain yield (PGY). Solid lines represent significant ($P < 0.05$) models fitted to N400 (A, C, G) or standardized data (B, D, F), and dashed lines represent significant ($P < 0.05$) models fitted to N0 data (A, C). Dotted lines represent the 1:1 relationship. Regression of mean values with $n = 36$ (12 genotypes × 3 experimental years) for each N level and regression of standardized data with $n = 72$ (12 genotypes × 2 N levels × 3 experimental years).

Table 4. Correlations (*r*) between standardized secondary traits of inbreds and plant grain yield (PGY) of hybrids, and path coefficients for direct and indirect (i.e., mediated by traits listed in the first column) effects of secondary traits of inbreds on PGY of hybrids.

Inbred traits†	Correlation with hybrid PGY		Direct effect on hybrid PGY	Indirect effects of§											
	r‡	*P*		TT_{SILK}	ASI	LAI_{PM}	$fIPAR_{PM}$	$IPARI_{GF}$	$IPARI_{PM}$	EGR_{CP}	EGR_{CP}/PGR_{CP}	KNE_1/EGR_{CP}	HI	NHI	NUE
TT_{ANT}	−0.22	ns#	0.75	0.58	0.34	0.32	0.32	0.18	0.37	−0.27	−0.04	−0.12	−0.42	−0.40	−0.38
TT_{SILK}	−0.40	<0.001	−0.18	—–	−0.17	−0.11	−0.12	−0.08	−0.12	0.06	0.02	−0.004	0.13	0.11	0.13
ASI	−0.44	<0.001	−0.54	−0.49	—–	−0.30	−0.36	−0.23	−0.33	0.12	0.04	−0.04	0.37	0.28	0.38
LAI_{MAX}	−0.15	ns	−0.89	−0.47	−0.45	−0.29	−0.39	−0.38	−0.58	−0.18	−0.05	0.08	0.39	0.20	0.37
LAI_{PM}	−0.48	<0.001	−2.21¶	−1.31	−1.24	—–	−2.11	−1.84	−1.55	1.43	1.32	−0.68	1.64	1.53	1.62
$fIPAR_{MAX}$	0.21	ns	−0.48	−0.05	0.01	0.04	0.03	−0.08	−0.19	−0.12	−0.12	0.16	−0.03	−0.07	−0.02
$fIPAR_{PM}$	−0.45	<0.001	2.20	1.47	1.48	2.10	—–	1.93	1.74	−1.16	−1.03	0.46	−1.79	−1.54	−1.76
$IPARI_{CP}$	0.22	ns	1.10	0.07	<−0.001	−0.24	−0.18	0.08	0.43	0.37	0.35	−0.27	0.12	0.26	0.07
$IPARI_{GF}$	−0.33	0.005	−0.05	−0.02	−0.02	−0.04	−0.04	—–	−0.04	0.02	0.02	−0.01	0.03	0.02	0.03
$IPARI_{PM}$	−0.27	0.024	−0.77	−0.50	−0.47	−0.54	−0.61	−0.64	—–	0.18	0.12	<−0.001	0.54	0.43	0.54
PGR_{CP}	0.06	ns	−0.14	0.02	−0.003	0.01	<0.001	−0.02	−0.02	−0.05	−0.003	−0.02	−0.02	−0.04	−0.02
$BIOM_{SILK}$	−0.18	ns	1.25	0.51	0.68	0.33	0.48	0.37	0.67	0.40	0.26	−0.18	−0.44	−0.12	−0.36
$BIOM_{PM}$	−0.21	ns	−2.12	−0.56	−1.00	−0.88	−1.11	−1.15	−1.11	−0.36	0.14	−0.43	0.57	0.15	0.56
RUE_{CP}	−0.09	ns	0.81	−0.19	−0.08	0.06	0.03	0.01	−0.19	0.02	−0.23	0.20	0.03	0.14	0.09
RUE_{GF}	0.06	ns	0.41	−0.11	−0.06	−0.05	−0.07	−0.05	−0.15	−0.02	−0.08	0.25	0.21	0.21	0.18
RUE_{PM}	−0.06	ns	−1.16	0.34	0.02	0.04	0.004	−0.02	0.19	−0.31	0.03	−0.33	−0.31	−0.37	−0.33
EGR_{CP}	0.50	<0.001	1.97	−0.65	−0.43	−1.28	−1.04	−0.90	−0.45	—–	1.69	−0.97	0.99	0.96	0.96
EGR_{CP}/PGR_{CP}	0.46	<0.001	−1.13	0.11	0.08	0.68	0.53	0.59	0.18	−0.97	—–	0.79	−0.39	−0.36	−0.38
KNP/PGR_{CP}	0.16	ns	1.13	−0.35	−0.33	−0.62	−0.60	−0.53	−0.44	0.60	0.52	0.22	0.80	0.61	0.75
KNE_1/EGR_{CP}	−0.36	0.002	−0.05	−0.001	−0.003	−0.02	−0.01	−0.01	<−0.001	0.02	0.03	—–	−0.01	−0.01	−0.003
HI	0.40	<0.001	−3.12	2.25	2.10	2.32	2.54	1.96	2.21	−1.58	−1.07	−0.37	—–	−2.64	−2.99
KNP	0.19	ns	−3.64	1.34	0.80	1.45	1.23	0.81	0.62	−2.50	−1.46	−0.74	−2.16	−1.97	−2.14
KW	0.04	ns	−0.06	0.01	0.002	−0.02	−0.002	−0.02	−0.002	0.01	0.03	−0.03	−0.01	−0.01	−0.01
PGY	0.20	ns	3.62	−1.59	−0.84	−0.85	−0.80	−0.21	−0.59	1.80	0.61	1.28	2.17	2.18	2.10
PN_{UPTAKE}	−0.14	ns	1.48	0.51	0.79	0.69	0.85	0.86	0.83	0.19	−0.08	0.26	−0.52	−0.29	−0.65
%Protein	−0.17	ns	0.74	0.45	0.45	0.40	0.45	0.48	0.54	−0.18	−0.13	0.06	−0.42	−0.35	−0.52
NHI	0.38	0.001	−0.92	0.54	0.47	0.64	0.64	0.47	0.52	−0.45	−0.29	−0.11	−0.78	—–	−0.75
$N/BIOM_{PM}$	0.02	ns	0.30	0.12	0.12	0.11	0.12	0.12	0.12	−0.04	−0.03	0.04	−0.10	−0.11	−0.17
NUE	0.36	0.002	3.04	−2.23	−2.15	−2.23	−2.44	−1.98	−2.16	1.49	1.02	0.19	2.91	2.49	—–

†Description in Table 1.

‡Correlation with $n = 72$ (12 genotypes × 2 N levels × 3 experimental years).

§Only for traits with significant correlation with hybrid PGY.

¶Underlined data indicate high direct and indirect effects (absolute path value ≥ 1).

#ns, not significant.

DISCUSSION

Trait Frequency Distribution and Phenotypic Plasticity

In the current research we explored a wide range of phenotypic variation in maize grain yield and its physiological and numerical components, both for hybrids and inbreds. The range was much larger for some of these traits in this species than in the only other work addressing parent–progeny relationships (Lee et al., 2005). For instance, variation with respect to mean values among hybrids evaluated in that work was 28% for PGY, 19% for $BIOM_{SILK}$, 26% for $BIOM_{PM}$, 27% for LAI_{MAX}, and 18% for HI, whereas in our study those percentages rose to 77, 53, 64, 67, and 32%, respectively. Certainly, a good part of the difference must be attributed to the wide range in N offer explored in our research as compared with the Canadian conditions (all their experiments were fertilized) and probably with other environmental effects (e.g., contrasting photothermal conditions associated with latitude and sowing date; Bonhomme et al., 1994; Otegui and Bonhomme, 1998), because general management practices were similar (e.g., stand density and row spacing) and other restrictions seemed to have minimum influence (e.g., pests or water deficit). However, the larger ranges obtained in Argentina held for most traits even when comparisons were limited to the N400 plots (30% for PGY, 27% for $BIOM_{SILK}$, 20% for $BIOM_{PM}$, 32% for LAI_{MAX}, and 31% for HI). Thus, failure in their capacity to establish genetic relationships between evaluated physiological components and in dissecting the

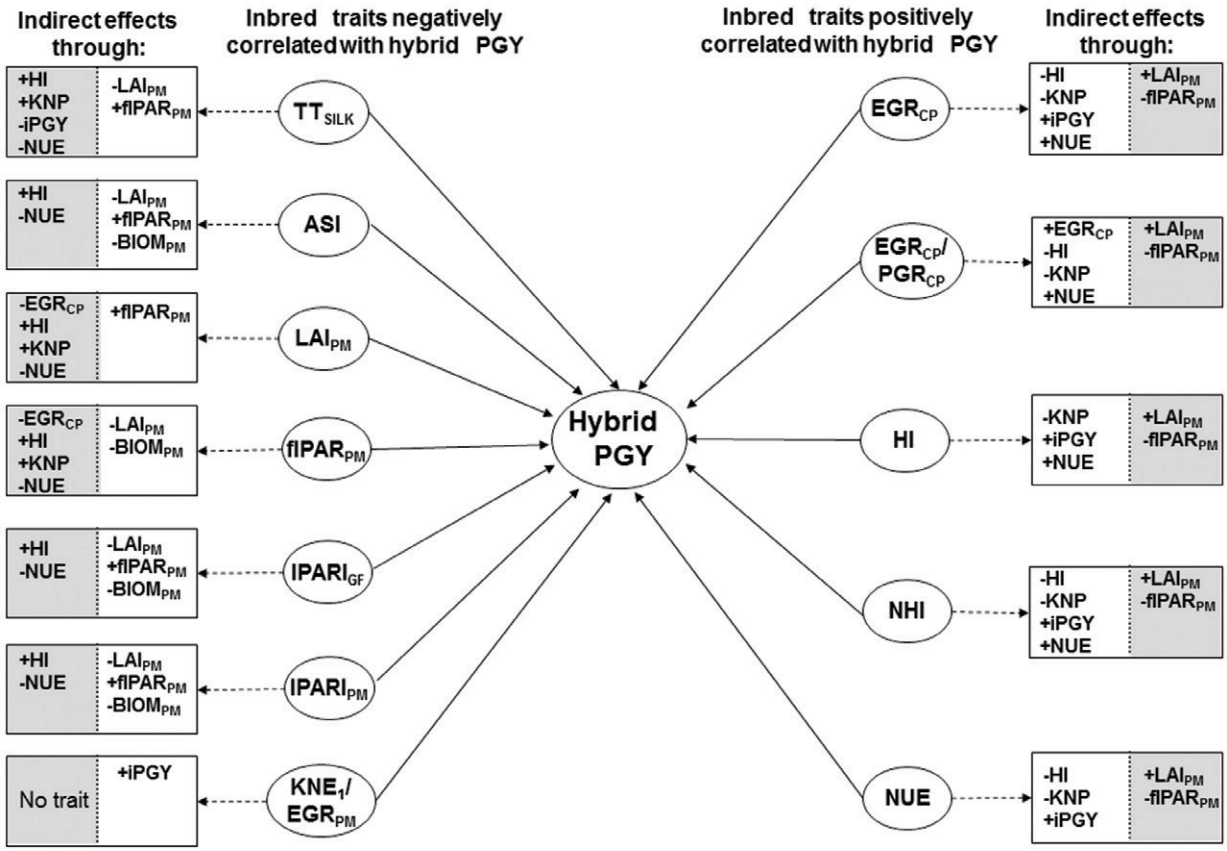

Figure 4. Conceptual representation of direct and indirect effects of traits measured in parental inbreds on hybrid PGY (abbreviation for inbred PGY is iPGY; the rest of the traits are described in Table 1). Inbred traits were grouped according to their significant ($P \leq 0.05$ for standardized values in Table 4) correlation with hybrid PGY (positive on the right and negative on the left). For each of these traits, represented within an ellipse, the direct effect on hybrid PGY is indicated by the solid arrow, and the indirect effects (i.e., mediated by other variable/s) on hybrid PGY are summarized in the adjacent box linked with a dashed arrow. Traits within each box have a positive (in white) or negative (in gray) correlation with the linked trait (e.g., LAI_{PM} and $fIPAR_{PM}$ are positively correlated with TT_{SILK}; HI, KNP, iPGY, and NUE are negatively correlated with TT_{SILK}). The sign (positive or negative) of each trait within a box indicates the sign of the indirect effect. For instance, the indirect effect of TT_{SILK} on hybrid PGY is (i) negative through LAI_{PM}, in spite of their positive correlation, and (ii) positive through HI, in spite of their negative correlation.

inheritance of grain yield was probably more related to the narrow range explored with each trait by Lee et al. (2005) than to statistical reasons raised by the authors. This limitation may be linked to a narrow genetic base for measured traits among inbreds included in their study (all elite short-season germplasm), which cannot be assessed because no phenotypic information is given for this genotypic group. In our research, ranges explored at N400 were always much larger for inbreds (63% for PGY, 39% for $BIOM_{SILK}$, 46% for $BIOM_{PM}$, 48% for LAI_{MAX}, and 43% for HI) than for hybrids. The failure may have also stemmed from the set of traits used for the analysis and its merit for a thorough interpretation of parent–progeny relationships conducive to elucidating the genetic controls subjacent to hybrid grain yield definition. About this aspect, several traits (e.g., all those related to the fraction and amount of light intercepted by the crop, growth rates during the critical period, radiation use efficiencies, grain yield components, and N-related traits) that are currently considered essential for the physiological understanding of grain yield determination in maize (Westgate et al., 2004; Lee and Tollenaar, 2007; D'Andrea et al., 2009) were completely lacking in previous genetic and breeding research of this species.

Important physiological traits were included in our study (Table 1) and phenotypic plasticity assessed within the framework proposed by Sadras and Slafer (2012), which is based on median and extreme percentile values. As expected and already commented on, the greatest part of the variation was driven by N conditions and not by genotypes (inbreds and hybrids did not differ markedly in the overall response pattern). In spite of the contrasting growing conditions caused by N (Table 2), no trait had a plasticity larger than 2 (i.e., 100% variation with respect to median values) as reported for all numeric components in small grain cereals (Sadras and Slafer, 2012), and variation in kernel numbers was limited to ~25% in hybrids and ~40% in inbreds. This is in agreement with the well-known reduced reproductive plasticity of a crop with decreased tillering capacity at normal stand densities and grain yield on the basis of a dominated female axillary inflorescence (Westgate et al., 2004), as compared with

tillering crops with apical inflorescences (e.g., winter cereals) or branching crops with flowers distributed on most parts of the plant (e.g., legumes) (Andrade, 1995). Independently of these considerations, plasticity of grain yield components decreased along the cycle (KNP > KW), as documented for other cereals (Sadras and Slafer, 2012). Lack of tillering may also be responsible for intermediate to low plasticity values found for LAI_{MAX} and its related traits $fIPAR_{MAX}$ and $IPARI_{CP}$. Interestingly, this trend shifted after flowering, and LAI_{PM} had one of the largest plasticity values among evaluated traits, with the concomitant effects on $fIPAR_{PM}$ and $IPARI_{GF}$. This response seemed chiefly effected by traits related to N metabolism, which were among those with largest plasticity (e.g., PN_{UPTAKE} and NUE) and known effects on leaf area duration (Sinclair and Horie, 1989; Uhart and Andrade, 1995). Conversely, most traits linked to radiation use efficiency and biomass production had low to intermediate plasticity, which in relative terms (i.e., as compared with leaf area duration) may be an expected response to N (Sinclair and Horie, 1989). These trends must be checked with data derived from experiments with variable growing conditions produced by other constraints (e.g., water deficit or stand density) or a combined effect of constraints.

Parent–Progeny Relationships

These relationships were evaluated for all measured traits by means of regression analysis, first of mean values within each N condition and next of standardized values. Except for a few traits (PGR_{CP} and KNP/PGR_{CP}), inbred performance was a good predictor of hybrid performance in at least one N condition, and in many cases (19 of 29 traits) the relationship held at both N levels. A similar trend was evident for standardized data (20 of 29 traits). As expected, traits related to plant development (TT_{ANT}, TT_{SILK}, and ASI) and KW always had tight parent–progeny relationships derived from their strong genetic control (Cross, 1975; Poneleit and Egli, 1979; Hallauer and Miranda Fo, 1988). Interestingly, this was also the case for many other physiological traits (LAI_{MAX}, $fIPAR_{MAX}$, $IPARI_{GF}$, $IPARI_{PM}$, $BIOM_{PM}$, EGR_{CP}/PGR_{CP}, HI, PN_{UPTAKE}, and NUE) for which there was no previous report on this topic in maize. Contrary to this group, several relationships set for mean values were exclusively driven by N and/or year effects and did not hold after standardization ($IPARI_{CP}$, PGR_{CP}, $BIOM_{SILK}$, RUE_{GF}, RUE_{PM}, KNE_1/EGR_{CP}, KNP, PGY, and $N/BIOM_{PM}$), whereas for others the parent–progeny relationship could be established for the whole data set only of standardized data (LAI_{PM}, $fIPAR_{PM}$, RUE_{CP}, EGR_{CP}, KNP/PGR_{CP}, and %Protein).

In agreement with previous reports (Betrán et al., 2003b), inbred grain yield was a good predictor of hybrid grain yield under optimal conditions (i.e., N400 plots) but not in the poor environment represented by N0 plots, and the relationship between these traits disappeared for standardized data. However, path analysis detected excellent surrogates for improving prediction of hybrid grain yield across environments among measured physiological traits. Some of them confirmed previous evidence from CIMMYT studies (Betrán et al., 2003a), as the negative correlation of hybrid grain yield with inbred TT_{SILK} (i.e., benefit of anticipated female flowering) and ASI (i.e., benefit of a reduced protandry). For others there was evidence from maize populations selected by CIMMYT for drought-prone environments (Edmeades et al., 1999), as the positive correlations with grain yield detected for EGR and EGR_{CP}/PGR_{CP} (i.e., benefit of increased biomass partitioning to the ear in inbreds) and HI (i.e., benefit of increased biomass partitioning to kernels in inbreds) as well as the lack of correlation with $BIOM_{PM}$ (i.e., no benefit of enhanced biomass accumulation along the cycle in inbreds). Interestingly, the positive correlation of hybrid PGY with inbred EGR can be attributed to large positive direct effects of the latter on the former, whereas for partitioning traits (EGR_{CP}/PGR_{CP}, HI, and NHI) it seems due to indirect effects mediated predominantly by the capacity for producing grains per unit of absorbed N (i.e., NUE). On one hand, these findings indicate that any attempt to increase hybrid grain yield through an enhanced HI among inbreds depends on the increase in inbred NUE. On the other hand, this avenue must be checked among elite maize germplasm because of the rather conservative nature of the carbon to N ratio of reproductive tissues as compared with the vegetative ones (Cazetta et al., 1999; D'Andrea et al., 2008), which conditions reproductive sink activity (D'Andrea et al., 2008). The scarse available evidence on this topic suggests this alternative may be already exhausted for some groups (Lee et al., 2005). About some N-related traits (NHI and NUE), current results are the first report on the benefit of their increase in inbreds for enhancing hybrid grain yield. Observed trends support the physiological advantage of increasing them for improving grain yield of any crop (Sinclair and Horie, 1989), provided this gain is not counterbalanced by a trade-off with grain quality and the concomitant penalty in final profits.

Finally, there was a group of traits measured in inbreds for which the correlation with hybrid grain yield was opposite to the expected result. This was the case of delayed senescence (LAI_{PM}), for which previous evidence (Betrán et al., 2003a) informed a positive response (i.e., benefit on hybrid grain yield of extended leaf area duration in parental inbreds) and current research a negative one. This apparent contradiction may have an explanation in the variation in phenology among inbreds included in our study (~200°Cd both for TT_{ANT} and TT_{SILK}, which represents ~14 d), and the positive correlation between TT_{SILK} and LAI_{PM} and ASI and LAI_{PM} detected among them. This variation was not excessively large in phenotypic plasticity terms, but it may have been large enough for exposing genotypes to variable growing conditions around silking (D'Andrea et al., 2009).

In other words, any possible benefit of delayed senescence could not compensate for the above-mentioned detrimental effects of delayed silking and increased protandry. This analysis also applies to other traits for which correlation with hybrids PGY differed from the expected trend (e.g., $fIPAR_{PM}$, $IPARI_{GF}$, and $IPARI_{PM}$) owing to their positive correlation with inbred LAI_{PM}. For avoiding this bias and having an enhanced assessment of leaf area duration (and related traits) effects on grain yield, future research should consider sowing at different dates for a more synchronous silking among genotypes.

CONCLUSIONS

In the current research we analyzed phenotypic plasticity and parent–progeny relationships of the main physiological determinants of maize grain yield and its components (kernel number and kernel weight), for which there is no previous thorough report. Environmental variation was caused by year effects and two contrasting N levels. We can highlight three major findings. First, and against our prediction, there were no marked differences between inbreds and hybrids in phenotypic plasticity of most traits; the largest part of the variation was caused by N levels rather than by genotypes. Second, there was a good (19 traits at both N levels) or acceptable (8 traits at one N level) predictive capacity of progeny phenotype (hybrids) on the basis of mid-parent phenotype (inbreds) for most evaluated traits; nevertheless, we demonstrated that in many cases (9 of the mentioned 27 traits) it was driven exclusively by environmental effects rather than by nonheterotic genetic effects (20 of the 29 traits). Fake parent–progeny relationships (i.e., environmentally driven) included traits such as grain yield and its main numeric determinant (kernel numbers) as well as PGR_{CP} and some RUEs (RUE_{GF} and RUE_{PM}). True parent–progeny relationships (i.e., genetically driven) included some traits related to light capture (LAI_{MAX} and $IPARI_{GF}$), total biomass production ($BIOM_{PM}$), biomass partitioning to reproductive organs (EGR_{CP}/PGR_{CP} and HI), N capture (PN_{UPTAKE}), and N use for grain production (NUE). Third, we identified 12 traits among inbreds that should allow improvement of hybrid grain yield independently of the environment. Two of these traits were particularly important (EGR_{CP} and NUE) because of their highly positive direct effect on hybrid grain yield as well as their high correlation with other traits that had a high indirect effect on hybrid grain yield (e.g., HI, EGR_{CP}/PGR_{CP}, ASI). Interestingly, true parent–progeny relationships were high ($b_2 > 0.5$) for all these traits.

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