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Heterosis × environment interaction in maize: What drives heterosis for grain yield?

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ABSTRACT

Variation in mean heterosis over a range of environments is expected when maize hybrids and inbred lines respond differently to environmental stimuli. However, the magnitude and nature of the heterosis × environment interaction (H × E) has not been adequately described. The objectives of this work were to determine (i) the effects of environmental variability on the expression of heterosis for plant grain yield (PGY) and related ecophysiological traits and (ii) to what extent H × E is of general importance for the expression of heterosis for these traits. Field experiments included a set of six inbred lines and twelve derived hybrids grown in 14 environments (year × nitrogen × water regime combinations) in the temperate region of Argentina. Main physiological and quantitative determinants of PGY were measured and mid parent heterosis (MPH) computed for each trait. Genotype × environment interaction was investigated using the joint regression analysis. For PGY, hybrids had a significant but moderate association between sensitivity to the environment and mean genotype value, whereas inbred lines did not show association. For harvest index (HI) hybrids showed greater mean values than inbreds, however, regression coefficients of both genotype groups tended to overlap slightly. A decrease in environmental quality led to a decline in the expression of heterosis for PGY but not for HI. A bilinear with plateau model adequately described the association between heterosis for PGY and environmental quality, because a threshold value was detected beyond which further increases in environment mean did not translate into higher heterosis for PGY. A similar response pattern was found between PGY MPH and biomass at physiological maturity ($Biomass_{PM}$) MPH. Despite the greater heterosis for $Biomass_{PM}$, further increases in PGY MPH could not be realized above a threshold value of 115 g pl^{-1} for $Biomass_{PM}$ MPH. HI MPH was the major factor that set a limit to PGY MPH under favorable environments.

1. Introduction

The complexity of grain yield as a quantitative trait comprises genetic and environmental components, which interact in often unpredictable ways. In maize (*Zea mays* L.), heterosis (i.e., hybrid vigour) is a key feature underlying the expression of grain yield in hybrids (Shull, 1909; East and Jones, 1919; Springer and Stupar, 2007; Troyer and Weillen, 2009). By definition, its magnitude depends on the relative performance of the hybrid to its parental inbred lines. Models of phenotypic expression include genetic and environmental effects. When interaction between genotypes (G) and environments (E) is present (i.e., change in rank of genotypes over a range of environments or a variation in the magnitude of response across environments) the phenotypic value of an individual includes also an interaction component, i.e. G × E (Falconer and Mackay, 1996). Under this premise it would be reasonable to define heterosis for grain yield in a specific environment as the sum of heterosis arising from genetic main effects and heterosis arising from G × E interaction effects.

Grain yield and grain yield heterosis result from complex interactions throughout development between physiological components that are dynamically influenced by the environment (Lippman and Zamir, 2007). These components are summarized in overall biomass production and its distribution between reproductive and vegetative tissues. The former is often described as the product between resource supply (light, water, nutrients) and the ability of plants for capturing a resource and converting it into biomass (Loomis and Connor, 1992), whereas the latter is usually

Abbreviations: PGY, plant grain yield; MPH, midparent heterosis; HI, harvest index; LAI_{MAX}, maximum leaf area index; RUE_{GF}, radiation use efficiency during the grain-filling period; $Biomass_{PM}$, final shoot plant biomass; KNP, kernel number per plant; KW, kernel weight.

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described as harvest index (HI, quotient between grain biomass and shoot biomass at maturity). The analysis of grain yield heterosis using this conceptual framework is recent (Tollenaar et al., 2004), and in most cases limited to a few genotypes and environments (Echarte and Tollenaar, 2006; Liu and Tollenaar, 2009a,b). However, a variation in mean heterosis over a range of environments is expected when hybrids and inbred lines respond differently to environmental stimuli (Munaro et al., 2011). In other words, the presence or absence of changes of heterosis across environments will be independent of the environment only if the single cross hybrid and parental inbred lines have the same sensitivity to the environment (i.e., same rate of change per unit variation in environmental quality). If a relationship (e.g., linear) can adequately describe the sensitivity to the environment of hybrids and inbred lines, changes in heterosis could be predicted from the relative sensitivities of hybrid and inbred lines to changes in the environment. The variable expression of heterosis in different environments must be approached within the topic of G × E interaction as variation depends not only on the genotype but also on the nature of the environmental variation. That is, heterosis × environment interaction (H × E) can vary according to the trait under study and its relation with fitness. As stated by Lippman and Zamir (2007), "... the magnitude of effect and significance of heterosis will vary between years, given the many phenotypes and loci involved and the influence on them of the environment". The magnitude of this variation has not been adequately quantified yet. An understanding of the effects of G × E on the expression of heterosis for grain yield and ecophysiological related traits could aid studies designed to assess the stability of quantitative trait loci (QTLs) affecting heterosis in maize under several stress conditions (LeDeaux et al., 2006). It could also assist our interpretation of the genetic controls behind these quantitative traits, which have not been unveiled so far (Lee et al., 2005). To our knowledge no study has been conducted in maize in which parental inbred lines and their derived hybrids have been grown at a sufficient number of environments so as to provide a range of conditions to evaluate sensitivity to the environment of both genotype groups and characterize the response of heterosis for grain yield and ecophysiological related traits across environments.

The purpose of this work is to report (i) the effects of environmental variability on the expression of heterosis for grain yield and ecophysiological traits in a set of single cross hybrids grown in 14 environments (year × nitrogen × water regime combinations) and (ii) to what extent G × E interactions are of general importance for the expression of heterosis for grain yield and ecophysiologically related traits.

2. Materials and methods

2.1. Genetic material and crop husbandry

Field experiments were conducted at the Pergamino Experimental Station of the National Institute of Agricultural Technology (INTA), Argentina (33°56′S, 60°34′W) on a Typic Argiudoll soil, during 2002–2003 (Exp. 1), 2003–2004 (Exp. 2), 2004–2005 (Exp. 3), 2006–2007 (Exps. 4 and 5) and 2008–2009 (Exps. 6 and 7). The genetic material evaluated included twelve 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, and LP561 × LP662. Inbred lines presented variability in breeding era, origin, canopy size, grain yield and grain yield components (D'Andrea et al., 2006). Lines also differed in the heterotic group of origin. Inbred B100 is US semi-dent germplasm (Hallauer et al., 1995) and the rest of the inbred lines belong to Argentine flint germplasm. Additionally, inbreds LP2 and LP561 were derived from Caribbean × Argentine germplasm. Treatments were a factorial combination of mentioned genotypes, and two N levels. These levels were a control with no added N (0 kg N applied) and a high N condition fertilized with 400 (Exps. 1–3) and 200 kg N ha^{-1} (Exps. 4–7). 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 consisted of 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}.

The variability among experiments was manipulated by applying supplemental irrigation or dry land farming. For Exps. 1, 2, 3, 4 and 6 supplemental irrigation was given to prevent water stress. Experiments 5 and 7 were conducted under dryland farming. Each combination of year × nitrogen × water regime was treated as a single environment, totaling 14 environments (Table 1). Further details on crop husbandry can be found in Munaro et al. (2011).

2.2. Measurements

Main physiological and quantitative determinants of grain yield were measured and their heterosis computed as described in Munaro et al. (2011). Those evaluated in the current analysis were (i) maximum leaf area index (LAI_{MAX}), (ii) radiation use efficiency during the grain-filling period (RUE_{GF}), (iii) final shoot plant biomass ($Biomass_{PM}$), (iv) kernel number per plant (KNP), (v) kernel weight (KW), (vi) harvest index (HI) and, (vii) plant grain yield (PGY). Briefly, LAI_{MAX} was calculated at silking as the product of green leaf area per plant and number of plants per unit land. RUE_{GF} was estimated as the quotient between biomass production and cumulative incident photosynthetically active radiation intercepted by the canopy between the onset of active grain filling (R_2, Ritchie and Hanway, 1982) and physiological maturity. Plants were individually harvested at physiological maturity. Plant material was oven dried at 60 °C for 7 days and weighed for final aboveground biomass determination (i.e., $Biomass_{PM}$). Each grained ear was individually hand-shelled, and kernel number was counted. Kernel number per plant was calculated by adding the kernels counted in apical and subapical ears (when present). Grain yield was computed for each harvested plant, and individual KW obtained as the quotient between PGY and KNP. For each treatment combination we computed mean values of HI, as the ratio between PGY and $Biomass_{PM}$.

2.3. Statistical analysis

For each environment, a randomized complete block design with three replicates was used for analysis. The occurrence of heterogeneity of error mean square was verified by Bartlett's test ($P < 0.05$) for variance comparisons. Assuming absence of significant reciprocal effects (D'Andrea et al., 2009), direct and reciprocal crosses of parental pair combinations (i.e., A × B and B × A) were pooled. Analysis of variance was performed across genotypes and environments to assess the significance of G × E interaction for all measured traits. Year × N × water regime combinations and genotypes were considered as random and fixed effects, respectively. The statistical significance of genotype, environment and G × E interaction was determined using the *F* test. Significant interactions were further investigated using the regression approach introduced by Yates and Cochran (1938) and developed by Finlay and Wilkinson (1963) and Eberhart and Russell (1966). The sensitivity to the environment of PGY and ecophysiological related traits for each genotype was calculated by regressing the mean trait value of individual genotypes on the mean trait value of all genotypes in each environment (i.e., environmental mean) but without the use

Table 1
Details of the environments used in the study and environmental mean trait value over all genotypes for a particular environment (i.e., environmental mean). Environments are ranked in according to decreasing mean plant grain yield (PGY). Maximum and minimum values of each trait are highlighted in bold.

Environment	Year	Applied N ($kg\,N\,ha^{-1}$)	Water	Environmental mean						
				PGY ($g\,pl^{-1}$)	KNP	KW (mg)	LAI_{MAX} ($m^2\,m^{-2}$)	RUE_{GF} ($g\,MJ^{-1}$)	$Biomass_{PM}$ ($g\,pl^{-1}$)‘	HI
1	2003–2004	400	Irrigated	**103.23**	**415**	242	3.94	1.82	**252**	0.40
2	2006–2007	200	Rainfed	98.03	381	244	4.19	NA	234	0.39
3	2006–2007	200	Irrigated	95.65	375	**246**	3.77	NA	193	**0.45**
4	2002–2003	400	Irrigated	91.2	367	240	3.81	1.94	231	0.38
5	2006–2007	0	Rainfed	82.97	341	237	3.31	NA	189	0.43
6	2008–2009	200	Irrigated	80.12	342	224	**4.33**	2.59	229	0.33
7	2008–2009	0	Irrigated	77.76	330	226	4.20	**2.67**	221	0.33
8	2004–2005	400	Irrigated	72.01	300	228	4.00	2.22	217	0.31
9	2004–2005	0	Irrigated	63.65	311	204	3.12	**1.38**	170	0.36
10	2003–2004	0	Irrigated	59.66	264	214	3.28	2.11	175	0.32
11	2002–2003	0	Irrigated	48.55	230	203	**2.73**	1.62	138	0.33
12	2006–2007	0	Irrigated	47.44	220	213	2.38	NA	**118**	0.37
13	2008–2009	0	Rainfed	30.73	203	145	3.64	2.06	134	0.21
14	2008–2009	200	Rainfed	**25.8**	**172**	**144**	3.95	2.03	140	**0.17**
			LSD	9.27	41.5	19	0.26	0.23	18.72	0.04

PGY: plant grain yield; KNP: kernel number per plant; KW: kernel weight; LAI_{MAX}: maximum leaf area index; RUE_{GF}: radiation use efficiency during active grain filling; $Biomass_{PM}$: shoot biomass at physiological maturity; HI: harvest index. NA: not available.

of log transformation. The analysis was based on the linear model as in Eq. (1):

$$Y_{ij} = u_i + b_i E_j + \sigma_{ij} \quad (1)$$

where Y_{ij} is the mean of ith genotype in jth environment, u_i is the mean of ith genotype over all environments, b_i is the regression coefficient measures the change in the mean performance of ith genotype per unit change in the mean of an environment, E_j is the environmental mean for jth environment, and σ_{ij} is the deviation from regression. The environmental mean (E_j) reflects a quantitative grading of all the environments from low to high, and was obtained as the average of all genotypes for each Year × N × water regime combination (i.e., 14 environments). The regression coefficient for each genotype allows a comparison to be made of the performance of each genotype with the average of all the genotypes. Regressions of unit slope ($b = 1$) have an average degree of response while those in excess of unity ($b > 1$) an above average degree of response, and vice versa. Slopes were tested for significant differences from unity (heterogeneity of regression lines) using t tests while the significance of the deviations from regression was tested by the F test based on pooled error. Analysis of variance and the joint regression were performed using Genes (2009). The proportion of G × E interaction explained by linear regression was defined as linear proportion. It is the ratio of sum of squares due to heterogeneity of regression to the total interaction sum of squares and indicates the relevance of linear regression of individual genotypes.

Heterosis was calculated for each experiment. Mid parent heterosis (MPH), i.e. the superiority of a hybrid compared to the parental mean, was calculated for each trait in absolute terms (Eq. (2)):

$$\mathrm{MPH} = F_1 - \mathrm{MP} \quad (2)$$

where F_1 is the mean of single-cross hybrids, and MP is the midparental value. Statistical significance of heterosis values for each trait was determined by t tests. For the study of heterosis × environment interaction (H × E) for grain yield and ecophysiological related traits, the environment mean was used on the abscissa for regression analysis. Logarithmic transformations were not considered as heterosis can often be removed by data transformation (Keller and Piepho, 2005) and the original scale is more understandable. For each trait, the MPH of each genotype was then regressed on the environmental mean. If the environment does not influence the heterotic response, then the slope of the linear function will be zero. If, however, changing the environment causes the slope to be significantly other than zero, then an environment dependent heterosis exists (i.e., H × E effect). The slope (b value) of the linear relationship measures the relative response of heterosis to varying environments. Linear and bilinear models were fitted by means of TBLCURVE (Jandel, 1992) and comparison of fitted models was done using the F-test.

Table 2
Mean square from the analysis of variance of plant grain yield (PGY), kernel number per plant (KNP), kernel weight (KW), maximum leaf area (LAI_{MAX}), radiation use efficiency during grain filling (RUE_{GF}), biomass at physiological maturity ($Biomass_{PM}$), and harvest index (HI).

Source	df	PGY	KNP	KW	LAI_{MAX}	$Biomass_{PM}$	HI	df	RUE_{GF}
Environment (E)	13	21,988**	200,456**	39,657**	12.06*	168,625**	0.213**	9	5.61**
Genotype (G)	11	30,435**	272,856**	41,200**	19.65**	96,286**	0.163**	11	3.44**
G × E	143	734**	7748**	1230**	0.33**	2125**	0.007**	99	0.41**
Heterogeneity of regression	11	5229**	19,996**	76,660**	1.34**	16,644**	0.014**	11	1.11**
Deviations	144	329*	6167*	637*	0.22 NS	839*	0.006*	88	0.29 NS
Error	308	129	2589	295	0.07	482	0.002	220	0.24
Linear proportion		55%	20%	48%	31%	60%	15%		30%

df: degrees of freedom; PGY: plant grain yield, KNP: kernel number per plant, KW: kernel weight; LAI_{MAX}: maximum leaf area index; $Biomass_{PM}$: shoot biomass at physiological maturity; HI: harvest index; RUE_{GF}: radiation use efficiency during active grain filling. NS: not significant.

* Significant at $P < 0.05$.

** Significant at $P < 0.01$.

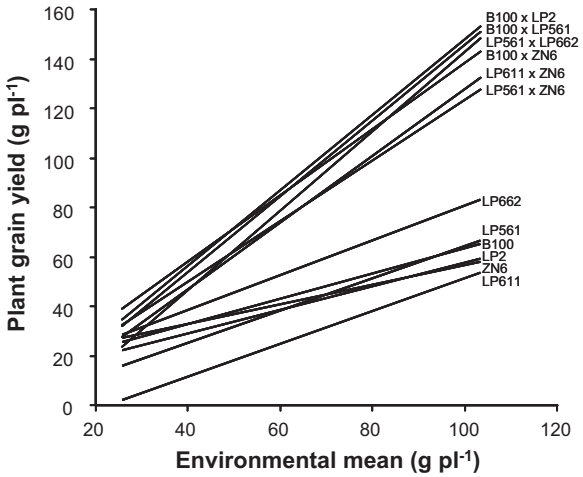

Fig. 1. Linear trends for PGY of inbred lines and derived hybrids regressed on the overall mean PGY of 14 environments. Mean values and regression coefficient (*b* value) as in Tables 3 and 4, respectively. Correlation coefficients were no less than 0.95 for hybrids and 0.77 for inbred lines.

3. Results and discussion

3.1. Environments and genotypes

The combined effect of interannual climatic variability and environmental manipulation (water and N) caused a wide range of responses in most of the evaluated traits (Table 1). The most severe environment (i.e., 2008–2009 rainfed and fertilized) reduced mean PGY across all entries by 75% with respect to their maximum value (2003–2004 irrigated and fertilized); a similar response was observed for HI, KNP and KW with reductions of 62, 59, and 41%, respectively. The inclusion of rainfed experiments allowed extending the environmental range to really adverse conditions for which threshold levels of zero yield were observed for inbred lines LP611 and LP561 (data not shown). Environmental means lower than those achieved in this study are rare. This type of environment limited the growth of both genotypic groups and inbred lines and hybrids had similar low yields under the most restrictive environments, while all other environments provided an opportunity for high performance of hybrids (Table 3). Few studies from CIMMYT achieved similar low grain yields of populations, inbreds or hybrids cultivated in tropical environments (Laffite et al., 1997; Betrán et al., 2003). Diversity with respect to environments was important to produce sufficient variability to quantify sensitivity to the environment of inbred lines and derived hybrids and response of heterosis across environments. In our study, the wide range in environmental mean for PGY (from 26 to 103 g pl^{-1}, Table 1), with fairly even distribution across the range, provided a robust basis for comparisons. G × E analysis is less meaningful if only high and low values of E_j are present but intermediate values are absent (Bernardo, 2010). Moreover, the number of environments in this study coincided with the threshold number of environments (i.e., at least 13) for assessing reliable slopes of joint regression when both potential and limited N treatments were carried out at the same location and in the same year (Zheng et al., 2009).

Among the genotypes, the highest mean PGY among inbreds corresponded to LP662 (i.e., 59.5 g pl^{-1}), and it was achieved through the highest values for KNP, $Biomass_{PM}$ and RUE_{GF} (301 grains pl^{-1}, 173 g pl^{-1} and 2.07 g Mj^{-1} d^{-1}, respectively; Table 3). In contrast, inbred LP611 had the lowest PGY (i.e., 31 g pl^{-1}) and this can be ascribed to low values of KNP, RUE_{GF} and HI (165 grains pl^{-1}, 1.31 g Mj^{-1} d^{-1} and 0.21, respectively). B100 had the highest mean HI among inbred lines (Fig. 1 and Table 3). Among hybrids, the highest mean value for PGY, KNP, RUE_{GF} and HI was for B100 × LP2, followed closely by hybrids that shared the same parental inbred line B100. Contrarily, hybrids LP561 × ZN6 and LP611 × ZN6 presented the lowest values for PGY, KNP, KW, RUE_{GF} and HI.

Hybrids involving B100 displayed high overall heterosis, although their specific response depended on the other inbred line included in the cross, whereas, hybrids not including B100 as a parent displayed variable overall heterosis regardless of environmental quality. In other words, the expression of high heterosis did not depend exclusively upon the use of B100 as a parental inbred, but its inclusion in the F1 cross guaranteed a high overall heterosis. Perhaps as a result of genetic divergence of parental inbred lines (Hallauer and Miranda, 1988), that is, US semi-dent and Argentine flint germplasm belonging to opposite heterotic groups that complement each other.

3.2. Genotype × environment interaction

For all traits, analysis of variance across genotypes and environments indicated significant ($P < 0.01$) G × E interactions (Table 2). When the interaction component was partitioned into linear and residual portions, the heterogeneity of regression mean square was significant ($P < 0.01$) for all traits and accounted for a relatively high proportion of the G × E interaction for $Biomass_{PM}$ and PGY (Table 2). For HI and KNP only a small proportion of the G × E interaction could be explained by heterogeneity of regression lines (15 and 20%, respectively). The contribution of differences in *b* value (heterogeneity of regression lines) to total interactions may be the result of a wide range of mean values across environments and genotypes having regression coefficients not confined to values close to 1 (Table 4). For PGY, $Biomass_{PM}$ and RUE_{GF} no inbred line had *b* values as high as those of hybrids (Table 4). Regression coefficients of inbred lines and hybrids, however, tended to overlap slightly for KNP and HI; that is, some inbred lines had *b* values similar to those of hybrids. The ranges of *b* values were large for all evaluated traits (Fig. 1 and Table 4), and varied by 92% for RUE_{GF} and 57% for HI. The response of the former could be attributed to the contrasting photosynthetic capacity and senescence between inbreds and hybrids during the grain-filling period (Ahmadzadeh et al., 2004), largely determined by N metabolism (D'Andrea et al., 2009). That of the latter reflects the limited plasticity in biomass allocation to reproductive organs in response to resource availability (Echarte and Andrade, 2003; Tollenaar and Lee, 2002). The remaining unpredictable part of variability of a genotype is related to deviation from overall regression. Eberhart and Russell (1966) proposed that deviations from the regression component of interaction for a genotype provided an additional useful stability parameter. No significant deviation was usually found for LAI_{MAX} and RUE_{GF} (Tables 2 and 5). Hybrids, on average, were more stable than inbreds (Table 5 and Fig. 2), particularly for HI. Differences among inbred lines in their stability or buffering capacity were observed for all traits (i.e., inbreds having significant and non significant deviations) except HI (Table 5 and Fig. 2).

The relative response of hybrids and inbred lines varied over environments, indicating a change in the superiority of one genotype over the others with respect to the environment (Fig. 1); however, no main crossover point (i.e., environment mean yield for which crossover interactions between genotypes reach the highest frequency) could be identified as a cut off to clearly divide environments. In practice, this implies that there was no single critical point to partition the environments into two groups (consisting of low-yielding and high-yielding environments), important for developing genotypes for specific adaptation. Nevertheless, when the G × E interaction for PGY was further analyzed through its physiological and numerical components, different association patterns

Table 3
Mean plant grain yield (PGY), kernel number per plant (KNP), kernel weight (KW), maximum leaf area (LAI_{MAX}), radiation use efficiency during grain filling (RUE_{GF}), biomass at physiological maturity ($Biomass_{PM}$), and harvest index (HI) of the 12 genotypes. Means are given for each genotype across environments.

Genotype	PGY ($g\,pl^{-1}$)	KNP	KW (mg)	LAI_{MAX} ($m^2\,m^{-2}$)	RUE_{GF} ($g\,MJ^{-1}$)	$Biomass_{PM}$ ($g\,pl^{-1}$)	HI
Inbred							
B100	48.3	229	205	2.47	1.93	132	0.35
LP2	42.0	252	168	3.02	1.60	125	0.32
ZN6	44.8	240	186	2.79	1.94	146	0.31
LP561	44.1	215	198	2.95	1.84	157	0.27
LP662	59.5	301	193	3.53	2.07	173	0.32
LP611	31.4	165	174	3.77	1.31	137	0.21
F_1 hybrids[a]							
B100 × LP2	101.7	402	240	4.17	2.38	222	0.43
B100 × ZN6	98.0	386	247	3.81	2.35	228	0.42
B100 × LP561	99.4	366	261	3.94	2.38	235	0.41
LP561 × ZN6	86.3	349	239	4.18	2.26	232	0.37
LP611 × ZN6	87.2	372	226	4.80	2.16	241	0.35
LP561 × LP662	94.4	373	242	4.03	2.34	243	0.37

PGY: plant grain yield, KNP: kernel number per plant, (KNP), kernel weight (KW), maximum leaf area (LAI_{MAX}), radiation use efficiency during grain filling (RUE_{GF}), biomass at physiological maturity ($Biomass_{PM}$), and harvest index (HI). Means are given for each genotype across.
[a] Pooled values direct and reciprocal crosses.

Table 4
Relative responsiveness to environmental change (*b* value) and standard error for plant grain yield (PGY), kernel number per plant (KNP), kernel weight (KW), maximum leaf area (LAI_{MAX}), radiation use efficiency during grain filling (RUE_{GF}), biomass at physiological maturity ($Biomass_{PM}$), and harvest index (HI) of the 12 genotypes.

Genotype	PGY		KNP		KW		LAI_{MAX}		RUE_{GF}		$Biomass_{PM}$		HI	
	b value	SE (±)	*b* value	SE (±)	*b* value	SE (±)	*b* value	SE (±)	*b* value	SE (±)	*b* value	SE (±)	*b* value	SE (±)
Inbred														
B100	0.51*	0.10	0.74*	0.14	0.31*	0.12	0.35*	0.12	0.45*	0.41	0.47*	0.11	0.65*	0.14
LP2	0.47*	0.11	0.83	0.21	0.32*	0.09	0.71	0.16	0.14*	0.35	0.34*	0.12	0.97	0.25
ZN6	0.39*	0.09	0.49*	0.17	0.57*	0.07	0.66*	0.12	0.56	0.23	0.54*	0.09	0.71*	0.17
LP561	0.65	0.16	0.66*	0.25	1.13	0.18	0.67*	0.08	0.62	0.21	0.61*	0.1	1.09	0.26
LP662	0.70	0.17	0.94	0.19	0.63	0.19	1.02	0.16	1.14	0.26	0.82	0.14	0.82	0.21
LP611	0.66*	0.12	1.08	0.17	0.99	0.14	1.05	0.17	0.50*	0.25	0.56*	0.1	1.51*	0.19
F_1 hybrids[a]														
B100 × LP2	1.52*	0.09	1.31*	0.10	1.27*	0.08	1.26*	0.1	1.21	0.23	1.29*	0.04	1.15	0.11
B100 × ZN6	1.33*	0.10	0.96	0.14	1.37*	0.13	1.25	0.11	1.76*	0.19	1.35*	0.11	0.82*	0.07
B100 × LP561	1.53*	0.10	1.18	0.15	1.42*	0.05	1.22*	0.09	1.47*	0.22	1.50*	0.07	1.09	0.11
LP561 × ZN6	1.22*	0.09	0.92	0.12	1.37*	0.07	1.23	0.11	1.50*	0.17	1.41*	0.08	0.80*	0.16
LP611 × ZN6	1.35*	0.10	1.26	0.14	1.28*	0.05	1.40*	0.16	1.37	0.28	1.45*	0.14	1.24*	0.07
LP561 × LP662	1.61*	0.07	1.55*	0.11	1.30*	0.08	1.15	0.11	1.18	0.21	1.58*	0.08	1.18*	0.09

Plant grain yield (PGY), kernel number per plant (KNP), kernel weight (KW), maximum leaf area (LAI_{MAX}), radiation use efficiency during grain filling (RUE_{GF}), biomass at physiological maturity ($Biomass_{PM}$), and harvest index (HI) of the 12 genotypes.
[a] Pooled values of direct and reciprocal crosses.
* Significantly different from $b = 1$ at $P < 0.05$.

Table 5
Significance of deviations from regression of plant grain yield (PGY), kernel number per plant (KNP), kernel weight (KW), maximum leaf area (LAI_{MAX}), radiation use efficiency during grain filling (RUE_{GF}), biomass at physiological maturity ($Biomass_{PM}$), and harvest index (HI).

Genotype	Deviation from regression						
	PGY	KNP	KW	LAI_{MAX}	RUE_{GF}	$Biomass_{PM}$	HI
Inbred							
B100	*	NS	*	NS	**	*	*
LP2	*	**	NS	NS	*	**	**
ZN6	NS	**	NS	NS	NS	NS	**
LP561	**	**	*	NS	NS	NS	**
LP662	**	***	*	NS	NS	**	**
LP611	**	**	*	NS	NS	NS	**
F_1 hybrids[a]							
B100 × LP2	NS	NS	NS	NS	NS	NS	NS
B100 × ZN6	*	*	*	NS	NS	*	NS
B100 × LP561	*	*	NS	NS	NS	NS	NS
LP561 × ZN6	NS	NS	NS	NS	NS	NS	**
LP611 × ZN6	NS	*	NS	NS	NS	**	NS
LP561 × LP662	NS	NS	NS	NS	NS	NS	NS

Plant grain yield (PGY), kernel number per plant (KNP), kernel weight (KW), maximum leaf area (LAI_{MAX}), radiation use efficiency during grain filling (RUE_{GF}), biomass at physiological maturity ($Biomass_{PM}$), and harvest index (HI).
[a] Pooled values direct and reciprocal crosses.
* Significant at $P < 0.05$.
** Significant at $P < 0.01$.

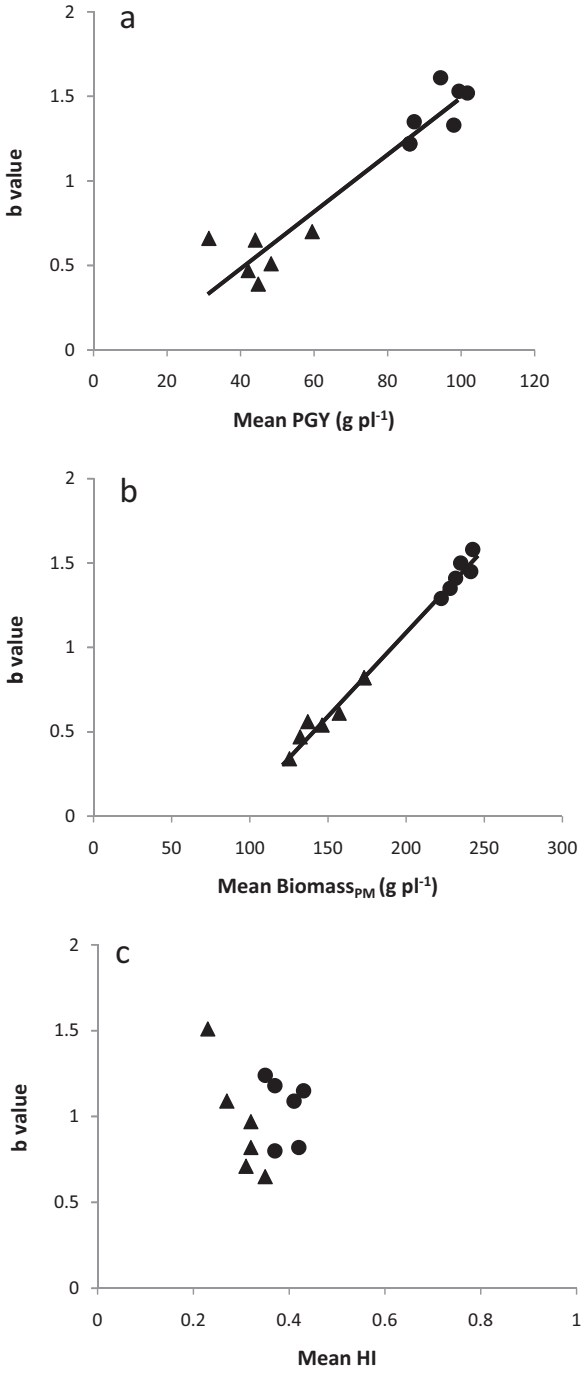

Fig. 2. Regression of *b* values (measure of sensitivity to environment) of inbred lines and derived hybrids on mean (i.e., overall mean across environments) performance for (a) plant grain yield (PGY), (b) biomass at physiological maturity ($Biomass_{PM}$) and (c) harvest index (HI). Solid circles are F_1s and solid triangles are inbred lines.

between *b* values and genotype means were observed (Fig. 2). A distinct linear trend in the regression between them (e.g., the greater the mean trait value the greater the sensitivity) is an indication of a tendency for convergence of regression lines to a single point (Mandel, 1961). A priori, a linear trend would be expected for most traits as a result of hybrids having higher mean values and greater capacity to capture the benefits of high yielding environments than inbred lines. Caution, however, must be exercised, as these associations may not hold within each genotype group across all traits. On the one hand, $Biomass_{PM}$ (Fig. 2b) and LAI_{MAX} (data not shown) had a highly significant and strong association ($r^2 > 0.93$, $P < 0.01$)

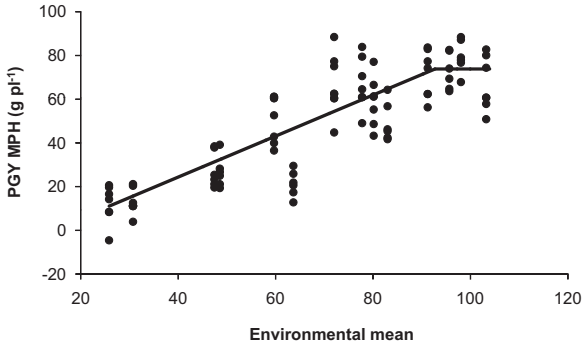

Fig. 3. Association between mid parent heterosis for plant grain yield (PGY) and environment mean. The bilinear fitted model (solid line) PGY MPH = 0.94*x* – 13.21 for $x < 92.7$ and PGY MPH = 73.84 when $x > 92.7$ ($r^2 = 0.79$, $n = 84$, $P < 0.01$).

between mean performance and linear sensitivity regardless of genotype group (inbred lines or hybrids). For these traits, significant heterogeneity of regression lines results from regression lines converging at an environment mean outside the observed range and radiating out from this region with varying slopes; non-parallel but non-intersecting lines resulting in differential change of mean but not of ranking of different genotypes That is, the position of the crossover point (point of intersection of the regression lines) lies beyond the observed environmental mean range. Alternatively, heterogeneity of regression lines may arise from non-parallel intersecting lines, as for HI and KNP. On the other hand, PGY (Fig. 2a) of hybrids had a significant but moderate association between sensitivity to the environment and mean genotype value ($r^2 = 0.42$, $P < 0.05$), whereas inbred lines did not show association ($r^2 = 0.01$, $P > 0.1$); i.e., hybrids have higher PGY mean values and this trait responds more strongly to increases in environmental quality than that of inbreds. For traits related to biomass partitioning like HI, which is highly associated with KNP (D'Andrea et al., 2006), hybrids showed greater mean values than inbreds. However, environmental sensitivities of both genotype groups displayed overlapping values (Fig. 2c). This could result in a constant value of heterosis for HI across the environments tested in this study. Apparently, for the set of environments studied here, the moderate association between PGY mean values and sensitivity to the environment found for hybrids could result from $Biomass_{PM}$ making a greater relative contribution to grain yield than HI. This is in agreement with previous evidence on breeding effects in maize, which detected a larger contribution to final PGY of total plant biomass than of HI (Tollenaar, 1989; Luque et al., 2006).

3.3. Heterosis and environment

The magnitude of heterosis in environments where high yield potential is expressed can be taken as reference for the analysis of H × E interaction such that heterotic effects will be maximized. Plotting the average heterosis for PGY over different types of environments that represented a gradient from high to low-yielding conditions (Fig. 3) revealed a two-phase response pattern, for which the initial positive response was followed by a plateau (bilinear fit: $r^2 = 0.79$, $n = 84$, $P < 0.01$; Fig. 3). On one hand, the positive phase is in agreement with hybrids having a greater sensitivity to the environment compared to inbred lines (Echarte and Tollenaar, 2006; D'Andrea et al., 2009). On the other hand, the plateau is indicative of a threshold environmental mean above which a further improvement in the quality of the environment does not translate into increases in MPH for PGY. Similarly, an enhanced environmental quality led to an increase in the expression of heterosis for most physiological determinants of grain yield ($0.05 > P > 0.01$; Fig. 4), with the exception of HI ($r^2 = 0.03$, $P = 0.1$). Apparently, the

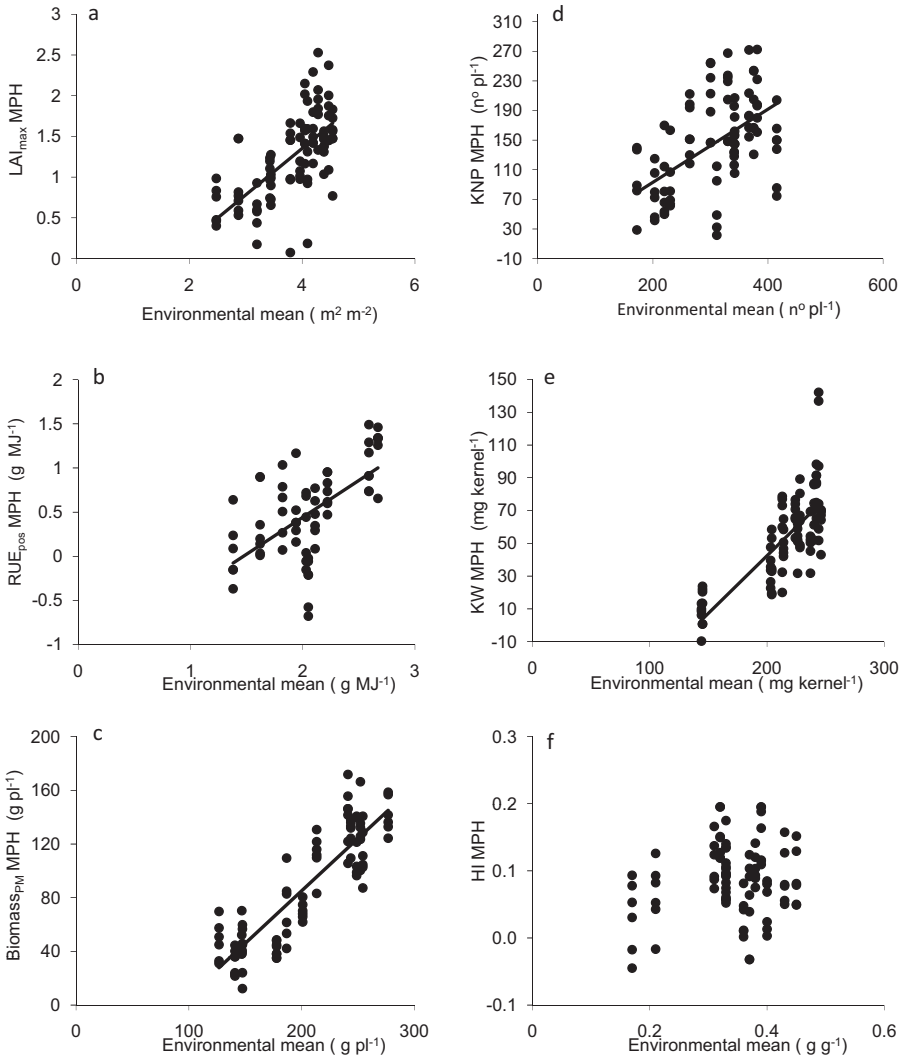

Fig. 4. Association between mid parent heterosis and environment mean for (a) maximum leaf area (LAI_{MAX}), (b) radiation use efficiency during grain filling (RUE_{GF}), (c) biomass at physiological maturity ($Biomass_{PM}$), (d) kernel number per plant (KNP), (e) kernel weight (KW), and (f) harvest index (HI). Fitted models were (a) LAI_{MAX} MPH = 0.57x − 0.93 (r^2 = 0.43, n = 84, P < 0.01), (b) RUE_{GF} MPH = 0.84x − 1.23 (r^2 = 0.38, n = 84, P < 0.01), (c) $Biomass_{PM}$ MPH = 0.78 − 71.28 (r^2 = 0.77, n = 84, P < 0.01), (d) KNP MPH = 0.49x − 5.81 (r^2 = 0.26, n = 84, P < 0.05), (e) KW MPH = 0.70 − 98.08 (r^2 = 0.60, n = 84, P < 0.01), and (f) HI MPH = 0.13x + 0.04 (r^2 = 0.03, n = 84, P = 0.1).

variability in HI MPH depended more on the genetic make up than on the response of HI to variation of the environments included in this study. The proportion of variation in MPH explained by variations in environmental mean was 77, 43, and 37% for $Biomass_{PM}$, LAI_{MAX} and RUE_{GF}, respectively. For the numerical components of PGY, 60% of the variation in MPH for KW could be explained by variations in environment mean, but only 26% for KNP.

The relationship between PGY and shoot biomass per plant (Vega et al., 2000; Echarte and Andrade, 2003), in which the slope of the regression of PGY on $Biomass_{PM}$ represents the rate of change of HI, is a useful framework to further analyze heterosis for PGY. Taking into account the immediate physiological determinants of PGY (i.e., $Biomass_{PM}$ and HI), we detected a response pattern that paralleled the association between MPH for PGY and environmental mean (Fig. 3). At high values of heterosis for $Biomass_{PM}$ (an indicator of environment quality), PGY MPH tended towards a plateau (Fig. 5a). In other words, the existence of a threshold value beyond which further increases in heterosis for $Biomass_{PM}$ did not translate into increases in heterosis for PGY was identified (bilinear fit: r^2 = 0.84, n = 84, P < 0.01). After removing the effects of MPH for $Biomass_{PM}$, the residuals were significantly (P < 0.01) associated with MPH for HI (Fig. 5b). That is, the scatter in heterosis for PGY was associated (r^2 = 0.48) with heterosis for HI. Apparently, for the set of genotypes used in current research, heterosis for HI placed an upper limit on the expression of heterosis for PGY. Despite the greater heterosis for $Biomass_{PM}$, further increases in PGY MPH could not be realized above a threshold value of 115 g pl^{-1} for $Biomass_{PM}$ MPH representative of favorable environments. In these conditions, HI MPH was the major factor that set a limit to PGY.

Trends in grain yield improvement in maize showed a marked increase with the exploitation of hybrid vigour through the development of hybrids, initially double crosses and subsequently single crosses (Crow, 1998). This improvement was associated with a greater HI of hybrids as compared to open pollinated varieties. The increase in partitioning of dry matter to the grains was the result of a decline in plant stature and a decrease in tassel size, among other traits (Duvick, 2005). Further increases in grain yield of newer hybrids were attained by reducing barrenness under high plant densities, as compared to older hybrids, and to a greater accumulation of biomass during the post flowering period due to a reduced rate of leaf senescence and functional stay green during grain filling (Bänziger et al., 2002), while HI per se has remained constant (Lorenz et al., 2010). It appears, however, that future possibilities and extent of exploitation of heterosis for PGY in breeding programs

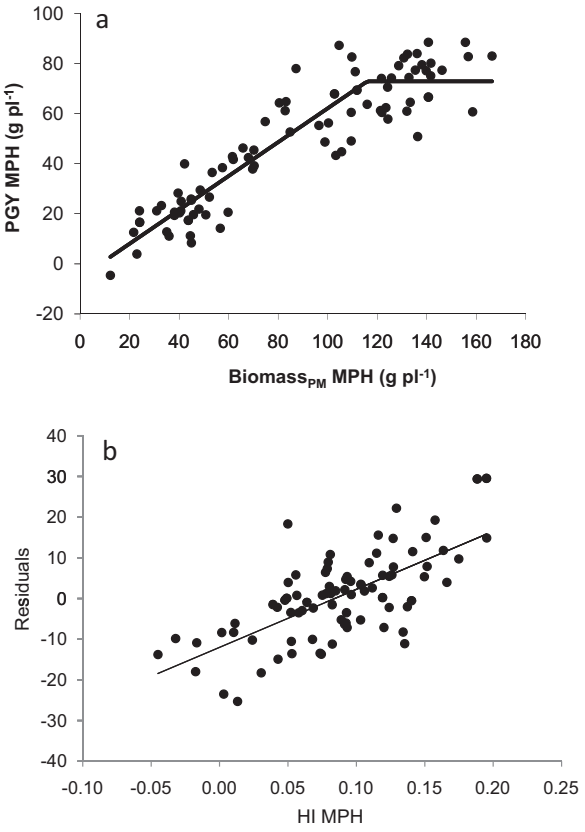

Fig. 5. Association between (a) mid parent heterosis for plant grain yield (PGY) and mid parent heterosis for $Biomass_{PM}$ and (b) residuals of regression in (a) and mid parent heterosis for HI. Fitted model in (a) PGY MPH = 0.67 – 5.69 for $x \leq 115$ and PGY MPH = 72.8 when $x > 115$ ($r^2 = 0.84$, $n = 84$, $P < 0.01$) and (b) residuals = 142.7HI MPH – 12.028 ($r^2 = 0.48$, $n = 84$, $P < 0.01$).

in temperate maize need to refocus on increasing HI per se. Under well-watered and N fertilized conditions, increasing the potential number of sinks per apical ear has been proposed as a possible path towards PGY improvement (Messina et al., 2009), therefore, a framework that allows identifying key variables related to biomass allocation to the ear as a function of plant growth rate during the critical period (Borrás et al., 2007) could aid plant breeders in selection for increased KNP at high plant growth rates.

4. Conclusions

Our study provided information on sensitivity to the environment for numerical and ecophysiological components of PGY of inbred lines and their derived hybrids. Numeric and ecophysiological components of grain yield showing significant heterosis, expressed either as per se or percentage MPH, had a positive linear relationship with the environment mean, with the exception of HI. An important finding is that heterosis for PGY in maize can be linearly related to environmental quality; however, a threshold value was identified above which no further increase in PGY MPH was observed. A similar response pattern was found between PGY MPH and BiomassPM MPH. Despite the greater heterosis for $Biomass_{PM}$, further increases in PGY MPH could not be realized above a threshold value of 115 g pl^{-1} for $Biomass_{PM}$ MPH representative of favorable environments. HI MPH was the major factor that set a limit to PGY. The magnitude and direction of the associations found in this study are a direct consequence of the genotypes and environments analyzed. Nevertheless, the relationships described here may prevail. Breeding programs in temperate maize need to refocus on increasing HI per se through selection for increased KNP at high plant growth rates as a possible path for future possibilities of exploitation of heterosis for BiomassPM and ultimately for PGY.

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References

Ahmadzadeh, A., Lee, E.A., Tollenaar, M., 2004. Heterosis for leaf CO_2 exchange rate during the grain-filling period in maize. Crop Sci. 44, 2095–2100.
Bänziger, M., Edmeades, G.O., Lafitte, H.R., 2002. Physiological mechanisms contributing to the increased N stress tolerance of tropical maize selected for drought tolerance. Field Crops Res. 75, 223–233.
Bernardo, R., 2010. Breeding for Quantitative Traits in Plants, second edition. Stemma Press, Woodbury, MN, p. 390.
Betrán, F.J., Beck, D., Bänziger, M., Edmeades, G.O., 2003. Genetic analysis of inbred and hybrid grain yield under stress and nonstress environments in tropical maize. Crop Sci. 43, 807–817.
Borrás, L., Westgate, M.E., Astini, J.P., Echarte, L., 2007. Coupling time to silking with plant growth rate in maize. Field Crops Res. 102, 73–85.
Crow, J.F., 1998. 90 years ago: the beginning of hybrid maize. Genetics 148, 923–928.
D'Andrea, K.E., Otegui, M.E., Cirilo, A.G., Eyherabide, G.H., 2006. Genotypic variability in morphological and physiological traits among maize inbred lines: nitrogen responses. Crop Sci. 46, 1266–1276.
D'Andrea, K.E., Otegui, M.E., Cirilo, A.G., Eyherabide, G.H., 2009. Ecophysiological traits in maize hybrids and their parental inbred lines: phenotyping of responses to contrasting nitrogen supply levels. Field Crops Res. 114, 147–158.
Duvick, D.N., 2005. The contribution of breeding to yield advances in maize (*Zea mays* L.). Adv. Agron. 86, 83–145.
East, E.M., Jones, D.F., 1919. Inbreeding and Outbreeding. J. Lippincott, Philadelphia, PA, p. 286.
Eberhart, S.A., Russell, W.A., 1966. Stability parameters for comparing varieties. Crop Sci. 6, 36–40.
Echarte, L., Andrade, F.H., 2003. Harvest index stability of Argentinean maize hybrids released between 1965 and 1993. Field Crops Res. 82, 1–12.
Echarte, L., Tollenaar, M., 2006. Kernel set in maize hybrids and their inbred lines exposed to stress. Crop Sci. 46, 870–878.
Falconer, D.S., Mackay, T.F.C., 1996. Introduction to Quantitative Genetics, fourth edition. Pearson Education Limited, Prentice Hall, Essex, England, p. 480.
Finlay, K.E., Wilkinson, G.N., 1963. The analysis of adaptation in a plant breeding programme. Aust. J. Agric. Res. 14, 742–754.
Genes, 2009. A Software in the Area of Genetics and Experimental Statistics. Departamento de Biologia Geral, Universidade Federal de Viçosa, Viçosa, Brasil.
Hallauer, A.P., Lamkey, K.R., Russell, W.A., White, P.R., 1995. Registration of B99 and B100 inbred lines of maize. Crop Sci. 35, 1714–1715.
Hallauer, A.P., Miranda, J.B., 1988. Quantitative Genetics in Maize Breeding, second edition. Iowa State Univ. Press, Ames, p. 468.
Jandel TBLCURVE, 1992. TableCurve 3.0. Curve Fitting Software. Jandel Scientific, Corte Madera, CA.
Keller, B., Piepho, H.P., 2005. Is heterosis an artifact governed by the choice of scale? Euphytica 145, 113–121.
Laffite, H.R., Edmeades, G.O., Taba, S., 1997. Adaptative strategies identified among tropical maize landraces for nitrogen-limited environments. Field Crops Res. 49, 187–204.
LeDeaux, J.R., Graham, G.I., Stuber, C.W., 2006. Stability of *QTL* involved in *heterosis* in *maize* when mapped under several stress conditions. Maydica 51, 151–167.
Lee, E., Ahmadzadeh, A.A., Tollenaar, M., 2005. Quantitative genetic analysis of the physiological processes underlying maize grain yield. Crop Sci. 45, 981–987.
Lippman, Z.B., Zamir, D., 2007. Heterosis: revisiting the magic. Trends Genet. 23, 60–66.
Liu, W., Tollenaar, M., 2009a. Physiological mechanisms underlying heterosis for shade tolerance in maize. Crop Sci. 49, 1817–1826.
Liu, W., Tollenaar, M., 2009b. Response of yield heterosis to increasing plant density in maize. Crop Sci. 49, 1807–1816.
Loomis, R.S., Connor, D.J., 1992. Crop Ecology: Productivity and Management in Agricultural Systems. Cambridge University Press, p. 538.
Lorenz, A.J., Gustafson, T.J., Coors, J.G., de Leon, N., 2010. Breeding maize for a bioeconomy: a literature survey examining harvest index and stover yield and their relationship to grain yield. Crop Sci. 50, 1–12.
Luque, S.F., Cirilo, A.G., Otegui, M.E., 2006. Genetic gains in grain yield and related physiological attributes in Argentine maize hybrids. Field Crops Res. 95, 383–397.

Mandel, J., 1961. Non-additivity in two-way analysis of variance. J. Am. Stat. Assoc. 56, 878–888.
Messina, C., Hammer, G., Dong, Z., Podlich, D., Cooper, M., 2009. Modeling crop improvement in a G × E × M framework via gene-trait-phenotype relationships. In: Sadras, V., Calderini, D. (Eds.), Crop Physiology: Applications for Genetic Improvement and Agronomy. Academic Press, London, pp. 235–265.
Munaro, E.M., D'Andrea, K.E., Otegui, M.E., Cirilo, A., Eyhérabide, G., 2011. Heterotic response for grain yield and ecophysiological related traits to nitrogen availability in maize. Crop Sci. 51, 1172–1187.
Ritchie, S.W., Hanway, J.J., 1982. How a Corn Plant Develops. Iowa State Univ. Special Report 48.
Shull, G.H., 1909. A pure line method of corn breeding. Am. Breeders Assoc. Rep. 5, 51–59.
Springer, N.M., Stupar, R.M., 2007. Allelic variation and heterosis in maize: how do two halves make more than a whole? Genome Res. 17, 264–275.
Tollenaar, M., 1989. Genetic improvement in grain yield of commercial maize hybrids grown in Ontario from 1959 to 1980. Crop Sci. 29, 1365–1371.
Tollenaar, M., Ahmadzadeh, A., Lee, E.A., 2004. Physiological basis of heterosis for grain yield in maize. Crop Sci. 44, 2086–2094.
Tollenaar, M., Lee, E.A., 2002. Yield potential, yield stability and stress tolerance in maize. Field Crops Res. 75, 161–170.
Troyer, A.F., Weillen, E.J., 2009. Heterosis is decreasing in hybrids. Yield test inbreds. Crop Sci. 49, 1969–1976.
Vega, C.R.C., Sadras, V.O., Andrade, F.H., Uhart, S.A., 2000. Reproductive allometry in soybean, maize and sunflower. Ann. Bot. 85, 461–468.
Yates, F., Cochran, W.G., 1938. The analysis of groups of experiments. J. Agric. Sci. 28, 556–580.
Zheng, B.S., Le Gouis, J., Dorvillez, D., Brancourt-Hulmel, M., 2009. Optimal numbers of environments to assess slopes of joint regression for grain yield, grain protein yield and grain protein concentration under nitrogen constraint in winter wheat. Field Crops Res. 113, 187–196.