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Classical Genetic and Quantitative Trait Loci Analyses of Heterosis in a Maize Hybrid Between Two Elite Inbred Lines

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ABSTRACT

The exploitation of heterosis is one of the most outstanding advancements in plant breeding, although its genetic basis is not well understood yet. This research was conducted on the materials arising from the maize single cross B73 × H99 to study heterosis by procedures of classical genetic and quantitative trait loci (QTL) analyses. Materials were the basic generations, the derived 142 recombinant inbred lines (RILs), and the three testcross populations obtained by crossing the 142 RILs to each parent and their F_1. For seedling weight (SW), number of kernels per plant (NK), and grain yield (GY), heterosis was >100% and the average degree of dominance was >1. Epistasis was significant for SW and NK but not for GY. Several QTL were identified and in most cases they were in the additive–dominance range for traits with low heterosis and mostly in the dominance–overdominance range for plant height (PH), SW, NK, and GY. Only a few QTL with digenic epistasis were identified. The importance of dominance effects was confirmed by highly significant correlations between heterozygosity level and phenotypic performance, especially for GY. Some chromosome regions presented overlaps of overdominant QTL for SW, PH, NK, and GY, suggesting pleiotropic effects on overall plant vigor.

THE term "heterosis" describes the superiority of heterozygous genotypes in one or more characteristics in comparison with the corresponding parental homozygotes (Shull 1908). The increased productivity of the heterozygotes, combined with their high fertility and resistance to biotic and abiotic stresses (Dobzhansky 1950), is exploited through the development of hybrid varieties in several crop species, and historically it represented one of the most revolutionary advancements in plant improvement. Despite a long dramatic history of successes, especially in maize (*Zea mays* L.) (Duvick 2001), there is still a striking discordance between an extensive agricultural practice of hybrid vigor utilization and our understanding of the basis of heterosis (Coors and Pandey 1999; Reif *et al.* 2006), and this hampers an effective exploitation of the phenomenon. Still, the production of new hybrids basically relies on empirical and time-consuming approaches (Duvick 2001). Dominance, real overdominance, and/or pseudo-overdominance and epistasis are the major genetic models invoked to explain hybrid vigor in the extensive scientific literature addressing heterosis in maize and other crops (Lamkey and Edwards 1999; Crow 2000; Reif *et al.* 2006; Lippman and Zamir 2007). The dominance hypothesis attributes increased vigor to the action at multiple loci of favorable dominant alleles from both parents combined in the hybrid (Bruce 1910; Jones 1917; Xiao *et al.* 1995; Cockerham and Zeng 1996). The overdominance hypothesis postulates instead the existence of loci at which the heterozygous state is superior to either homozygote (Shull 1908; East 1936; Crow 1948; Stuber 1994); "pseudo-overdominance" refers to a particular situation in which tightly linked genes with favorable dominant alleles in repulsion phase in the parental lines result in an apparent overdominance when combined in the hybrid (Crow 1952). Finally, the interaction of favorable alleles from the two parents at different loci, themselves showing additive, dominant, and/or overdominant actions, is taken into account by the epistasis hypothesis (Schnell and Cockerham 1992; Stuber *et al.* 1992; Li *et al.* 2001; Luo *et al.* 2001).

The application of molecular markers for germplasm evaluation (Brown and Kresovich 1996) and for the dissection of the genetic basis of many quantitative traits of economical importance in many crops (Edwards *et al.* 1992; Tanksley 1993; Paterson 1995; Kearsey and Farquhar 1998) prompted the development of two different approaches for the evaluation of heterosis on the basis of molecular markers. Genetic distance between parents, estimated by molecular markers, in fact

This article is dedicated to the memory of Ercole Ottaviano, geneticist and "maestro" for most of us.

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has been proposed as a useful tool for hybrid vigor prediction (Melchinger 1999). Several studies reported a positive correlation between genetic distance of parental lines and superior hybrid performance (Liu *et al.* 2002; Barbosa *et al.* 2003). An alternative approach, pioneered by Stuber *et al.* (1992), aims to identify and characterize the quantitative trait loci (QTL) contributing to heterosis, providing also indications about the genetic basis of the phenomenon. Several QTL for yield and/or yield components in maize were detected showing a variety of effects, including dominance, overdominance, and pseudo-overdominance (Stuber *et al.* 1992; Beavis *et al.* 1994; Ajmone Marsan *et al.* 2001; Lu *et al.* 2003).

Molecular data based on gene expression analysis by DNA microarrays comparing inbred lines and their corresponding F_1 hybrids in maize documented both additive and nonadditive gene expression levels (Swanson-Wagner *et al.* 2006). A contribution of true overdominance to heterosis is supported by gene expression levels documented also in diploid and triploid maize hybrids (Auger *et al.* 2005) and/or by an unusually high level of allelic transcription variation due to *cis*-regulatory elements independently reported in maize by Guo *et al.* (2004) and M. Morgante (unpublished data). It is therefore clear that additional research is required to obtain a deeper insight into the causal bases of heterosis from the perspective of developing novel and efficient breeding strategies for hybrid production. Here we present the results obtained by following approaches of both classical quantitative genetics and QTL analysis on genetic material developed from the single cross between maize inbred lines H99 and B73. Our research was conducted to (i) study the level of heterosis for traits of agronomic importance, (ii) detect the genetic effects involved (*i.e.*, allelic and nonallelic interactions) by both classical genetic and QTL analyses, (iii) investigate the relationships between the molecular marker heterozygosity and the phenotypic performance, and (iv) identify the genomic regions most involved in heterosis.

MATERIALS AND METHODS

Plant materials: The investigated materials were derived from the single cross B73 × H99. B73 belongs to the Iowa Stiff Stalk Synthetic (BSSS) heterotic group, whereas H99 was developed from Illinois Synthetic 60C and belongs to the Lancaster Sure Crop (LSC) heterotic group (Melchinger *et al.* 1991). Genotypes were represented by five groups, *i.e.*, the set of basic generations and four populations. Basic generations included the two parental inbreds, their two reciprocal F_1's, the F_2, and the two backcrosses (BCs) to B73 [BC(B)] and to H99 [BC(H)]. BC(B) and BC(H) were produced by using the F_1 as the male parent to avoid possible maternal effects in the early growth stages due to larger F_1 seeds. The four populations were (i) 142 *r*ecombinant *i*nbred *l*ines (RILs) obtained by single-seed descent from the original single cross after 12 selfing generations (F_{13}) and (ii) three testcross (TC) populations obtained by crossing the 142 RILs (as female parents) with B73 [TC(B)], H99 [TC(H)], and their F_1 [TC(F)]. Therefore, the three TC populations were produced following the triple testcross scheme (TTC) described by Kearsey and Jinks (1968) and Kearsey *et al.* (2003).

Field experiments: The five groups of materials (*i.e.*, the basic generations and the four populations) were field tested in 2002 at three random locations of the Po valley in northern Italy (Bologna, Cremona, and Milano). Each group of materials was arranged in separate but adjacent blocks; the five blocks were included in the same replication with two replications per location. The field layout was a randomized complete block design for basic generations, whereas it was a modified split-plot design for the four populations (Lu *et al.* 2003); the populations corresponded to the main plots and the RILs (either *per se* or combined with a tester) corresponded to the subplots. All materials were grown in single-row plots 4.40 m long and 0.80 m wide, including 22 plants after thinning (6.25 plants m^{-2}). In the basic generation trials, each plot was flanked by one border row at each side, accounting for the different heterozygosity levels; moreover, F_2 was entered twice because of its larger heterogeneity. For the four populations, main plots were separated by two pairs of border rows because of the different levels of plant vigor expected among populations. Current field practices for maize were used and three to four irrigations were provided (18–20 mm each) to attain favorable growing conditions. Basic generations and the RIL population were hand harvested and shelled when uniform moisture was achieved; the TTC populations were machine harvested and kernel moisture was measured soon after shelling.

Data were collected on a single-plot basis for the following traits: (1) percentage of seedling emergence (SE) 3 weeks after sowing; (2) seedling dry weight (SW) ~40 days after sowing (fifth-leaf stage) on a sample of six plants per plot, collected before thinning; (3) days to pollen shedding (PS), measured as the interval between sowing date and PS date (assessed when 50% of plants had extruded anthers); (4) anthesis-silking interval (ASI), the difference between silking date (when 50% of plants had extruded silks) and PS date; (5) plant height (PH), measured at the flag leaf collar on three competitive plants per plot, except for the F_2, BC(B), and BC(H) in the basic generations and the TC(F) population, for which six plants were examined per plot; (6) kernel moisture (KM) at harvest (for the TTC populations only); (7) grain yield (GY) adjusted to 15.5% moisture; (8) kernel weight (KW) adjusted to 15.5% moisture on a sample of 100 kernels; and (9) number of kernels per plant (NK), calculated as the ratio between grain yield per plant and KW. SE was evaluated in two trials only, whereas SW was measured in all three trials but in only one replication each.

Analysis of field data and of heterosis: For each group of materials, analysis of variance (ANOVA) was conducted on single-plot mean values within each trial (environment) and then combined across trials. For SE percentage, ANOVA was also conducted on data subjected to angular transformation (Steel and Torrie 1980); however, since the two analyses provided similar information, only results concerning original data are presented. The analyses were conducted using SAS GLM and VARCOMP procedures (SAS Institute 1996), and least-square means over locations were used for subsequent analyses. For the basic generations, *F*-test for the comparison among entries across trials was computed using genotype × environment interaction ($g \times e$) as denominator when it was significant. For the four populations, ANOVA was performed using a split-plot procedure, except that subplot treatments (*i.e.*, RILs *per se* or combined with a tester) were analyzed within main plots. The differences among the four populations were thus confounded with the differences among main plots. The

comparison among the four populations across trials was computed using population × environment interaction when significant. For each trait, the best-performing RIL was compared to the F_1 by using the Scheffe's test (Steel and Torrie 1980).

The percentage of heterosis was analyzed in the basic generations in two ways: (i) 100 × (F_1's mean − parents' mean)/parents' mean and (ii) by regressing the mean values across the two parents, the two F_2 entries, the two BCs, and the two F_1's on the corresponding heterozygosity level (*i.e.*, 0, 0.5, 0.5, and 1, respectively) and estimating heterosis as the ratio between slope (b_1) and intercept (b_0) of the regression.

Heterosis was also evaluated in the TC(B) and TC(H) populations. Midparental heterosis (H_{mp}) of each TC hybrid was calculated as follows: $TC\ H_{mp_i} = TC_i - MP_i$, where TC_i was the mean value of the TC_i hybrid and $MP_i = (RIL_i + \text{tester})/2$ was the midparental value of the corresponding RIL and the tester inbred line (*i.e.*, B73 or H99).

NCIII and TTC analysis: Following Kearsey and Pooni (1996) and Kearsey *et al.* (2003), the crosses of the $n = 142$ RILs to H99, B73, and the F_1 are indicated as L_{1_i}, L_{2_i}, and L_{3_i} ($i = 1 - 142$), respectively. TTC families were subjected to ANOVA to test for additive ($L_{1_i} + L_{2_i}$) and dominance ($L_{2_i} - L_{1_i}$) variation following the standard North Carolina III (NCIII) design and for epistatic variation ($L_{1_i} + L_{2_i} - 2L_{3_i}$) as described by Kearsey and Pooni (1996). Additive (V_A) and dominance (V_D) components of genetic variance were estimated and used to calculate the average degree of dominance, [as $\sqrt{(2V_D/V_A)}$], which is a weighted mean of the level of dominance over all segregating loci (Kearsey and Pooni 1996). The two independent sets of data obtained by summation ($L_{1_i} + L_{2_i}$) and by subtraction ($L_{2_i} - L_{1_i}$) of TC(B) and TC(H) values hereafter are indicated as the "SUM" data set and the "DIFF" data set, respectively. Variation within the SUM data set and within the DIFF data set is due to additive and dominance effects, respectively, combined over TC(B) and TC(H) populations.

Genetic linkage map: Our RIL population was the reference population for mapping purposes. This population was previously genotyped and used for the production of a genetic linkage map (Sari-Gorla *et al.* 1997; Frova *et al.* 1999). This map was then edited and implemented with additional microsatellite markers (simple sequence repeats). Population genotyping was achieved according to the protocol available at http://www.maizegdb.org/documentation/maizemap/ssr_protocols.php, with minor modifications. New markers were either added to the existing frame or were used to replace markers, mostly RFLPs with missing data. A total of 158 loci were arranged in a genetic linkage map (B73/H99 RI 2005, available at http://www.maizegdb.org) using the MAPMAKER3 program (Lander *et al.* 1987).

Analysis of main-effect QTL: Composite interval mapping (CIM) (Zeng 1994) was used to identify QTL with the software PLABQTL (Utz and Melchinger 1996). Cofactors were selected by stepwise regression with an "F-to-enter" and an "F-to-delete" value of 3.5. The identification of QTL was performed in two steps. In the first step, model 1 (Zeng 1994) was fitted using the selected markers as cofactors to the extent that they were unlinked to the genomic region under search; then, in a second step, model 2 (Zeng 1994) was used to confirm detected QTL by including as cofactors also markers linked to the tested region. A QTL was declared present when its LOD value exceeded the threshold with model 1 and a peak was also detected with model 2 or when LOD was significant under model 2 but not detected with model 1 (*e.g.*, because of a linked QTL). Estimates of QTL positions were obtained at the point where the LOD score reached its maximum with model 2 in the region under consideration. LOD (= 0.217 likelihood ratio) threshold for declaring a putative QTL for each trait, data set, and model was defined by 1000 permutations (Churchill and Doerge 1994). The QTL effect was defined as 1/2(H99 − B73), and therefore it was positive when H99 allele was associated with the highest value. QTL detected with different populations or for different traits were considered as common if their estimated map position was within a 20-cM distance (Groh *et al.* 1998). The proportion of variance explained by all QTL was determined by the adjusted coefficient of determination of regression (R^2_{Adj}), fitting a model including all detected QTL.

Analyses were carried out on mean values over locations of RILs and TC(F) populations as well as on TC(H) H_{mp}, TC(B) H_{mp}, SUM, and DIFF data sets (Table 1). In the absence of epistasis, the analysis of RILs, TC(F), and SUM data set identified QTL on the basis of their additive effects (a), whereas the analysis of TC(H) H_{mp}, TC(B) H_{mp}, and DIFF data sets identified QTL on the basis of their dominance effects (d). In fact, the (QQ *vs.* qq)/2 contrast has the expectation of $c_0 = 2a/2$ in the RIL population, of $c_1 = (a - d)/2$ in TC(H), and of $c_2 = (d + a)/2$ in TC(B). Therefore, the (QQ *vs.* qq)/2 contrast in the SUM data set has the expectation of $c_1 + c_2$, which is a direct estimate of a, whereas in the DIFF data set the (QQ *vs.* qq)/2 contrast has the expectation $c_2 - c_1$, which is a direct estimate of d. The same contrast, in both TC(H) H_{mp} and TC(B) H_{mp} data sets, has the expectation of $d/2$.

The degree of dominance of a QTL was estimated as $|d/a|$. For this purpose, for all QTL declared as significant within any data set, dominance and additive effects were estimated in SUM and DIFF data sets. These estimates were used to calculate $|d/a|$ and to classify the QTL as additive ($|d/a| < 0.2$), partially dominant ($0.2 \le |d/a| < 0.8$), dominant ($0.8 \le |d/a| < 1.2$), and overdominant ($|d/a| \ge 1.2$) according to Stuber *et al.* (1987).

Analysis of epistatic QTL: A mixed linear model was used to confirm main-effect QTL found in the previous analysis and to map digenic epistatic QTL in the SUM and DIFF data sets. For this purpose, we used QTLMapper (Wang *et al.* 1999), which allows simultaneous interval mapping of both main-effect and digenic epistatic QTL in a data set with two possible genotypes at each marker locus. The analysis was first conducted without including epistasis to confirm the QTL detected with the method previously described; then, the analysis was conducted including epistasis in the model. In both analyses, QTL mapping was carried out in three steps. First, significant markers were identified by the mean of stepwise regression based on single-marker genotypes for putative main-effect QTL and, on all possible marker pairs, for putative epistatic QTL. Then, QTL were detected using CIM in genomic regions identified in the first step. A threshold of $P \le 0.001$ and $R^2 > 5\%$ was used (Wang *et al.* 1999; Li *et al.* 2001). Finally, effects and test statistics associated with significant main-effect and epistatic QTL were obtained using the restricted maximum-likelihood estimation method, as described by Wang *et al.* (1999).

Genetic expectations of the parameters estimated in the epistatic models differ according to genetic composition of data sets analyzed. For the SUM data set, the estimated interaction is expected to be predominantly of the additive × additive type, whereas for the DIFF data set it is expected to be predominantly of the dominance × dominance type.

Relationship between molecular marker heterozygosity and phenotypic performance: Relationship between molecular marker heterozygosity and phenotypic performance was tested by regressing phenotypic performance on genome heterozygosity in both TC(B) and TC(H) populations; TC(F) was not considered since its heterozygosity level is expected to be the same (*i.e.*, 50%) for all 142 entries. For each RIL, we calculated the percentage of total marker loci homozygous for the allele

TABLE 1

Genotypes and genotypic values of populations and data sets

Population and data set[a]	RIL genotype[b]	Genotype of the population/data set	Genotypic value[c]	Half of the QQ *vs.* qq contrast [(QQ − qq)/2]:
RIL	QQ	QQ	$P + a$	
	qq	qq	$P - a$	$c_0 = (2a)/2 = a$
TC(B)	QQ	Qq	$P + d$	
	qq	qq	$P - a$	$c_1 = (a + d)/2$
TC(H)	QQ	QQ	$P + a$	
	qq	Qq	$P + d$	$c_2 = (a - d)/2$
TC(F)	QQ	$\frac{1}{2}$QQ + $\frac{1}{2}$Qq	$P + \frac{1}{2}(a + d)$	
	qq	$\frac{1}{2}$Qq + $\frac{1}{2}$qq	$P + \frac{1}{2}(d - a)$	$c_3 = a/2$
SUM	QQ	QQ + Qq	$2P + a + d$	
	qq	Qq + qq	$2P - a + d$	$c_{\text{SUM}} = (2a)/2 = a$
TC(B) H_{mp}	QQ	Qq − $\frac{1}{2}$(QQ + qq)	$d - \frac{1}{2}(a - a)$	
	qq	qq − $\frac{1}{2}$(qq + qq)	$-a - \frac{1}{2}(-2a)$	$c_{\text{TC(B) } H_{\text{mp}}} = d/2$
TC(H) H_{mp}	QQ	QQ − $\frac{1}{2}$(QQ + QQ)	$a - \frac{1}{2}(a + a)$	
	qq	Qq − $\frac{1}{2}$(qq + QQ)	$d - \frac{1}{2}(-a + a)$	$c_{\text{TC(H) } H_{\text{mp}}} = -d/2$
DIFF	QQ	QQ − Qq	$a - d$	
	qq	Qq − qq	$a + d$	$c_{\text{DIFF}} = (-2d)/2 = -d$

[a] Populations are the RILs, their corresponding testcrosses to B73 parent [TC(B)], to H99 parent [TC(H)], and to the F_1 hybrid [TC(F)]. Data sets were obtained by the sum TC(B) + TC(H) [SUM], the difference TC(H) − TC(B) [DIFF], the midparental heterosis (H_{mp}) of each TC(B) [TC(B) H_{mp} = TC(B) − MP, where MP = (RIL + B73)/2] and each TC(H) [TC(H) H_{mp} = TC(H) − MP, where MP = (RIL + H99)/2].

[b] Q, QTL allele of H99; q, QTL allele of B73.

[c] P, mean of the two QQ and qq homozygotes; a, additive effect; d, dominance effect. a has been defined as $\frac{1}{2}$(H99 − B73), and it is positive when H99 has the highest value.

of one parental line, which can be considered as an estimate of the percentage of the total genome originating from that parental inbred (genome ratio). Then, the genome heterozygosity of one TC(B) hybrid was equal to the percentage of H99 genome in the corresponding RIL, while the genome heterozygosity of one TC(H) hybrid was equal to the percentage of B73 genome in the corresponding RIL. The effect of heterozygosity on the phenotypic performance was investigated by following three different approaches, which varied on the basis of the dependent variable used in the regression analysis. The dependent variables were (i) performance *per se* of each hybrid in TC(B) and TC(H) populations, (ii) heterosis effects as obtained in TC(H) H_{mp} and TC(B) H_{mp} data sets, and (iii) heterosis effects as obtained in the DIFF data set. When the DIFF data set was used as a dependent variable, genome heterozygosity of the TC(H) hybrids was the independent variable since the DIFF data set was calculated as TC(H) − TC(B).

RESULTS

Basic generations and heterosis: ANOVA (not shown) revealed that differences among trials (environments) and genotype × environment ($g \times e$) interaction were significant ($P \leq 0.05$) or highly significant ($P \leq 0.01$) for most traits. However, in all instances the variance due to genotypes was much greater than the $g \times e$ variance; therefore, only mean values across trials are presented and discussed.

The two parental inbreds differed for several traits (Table 2), and H99 showed significantly lower mean values than B73 for PS, PH, GY, and NK. Differences between the two reciprocal crosses were always negligible (data not shown) and F_1 mean value for GY was high (10.69 Mg ha^{-1}). Comparison between the F_1 mean and the parental mean was not significant for ASI, was significant for SE, and was highly significant for all other traits. F_1 mean was lower than the parental mean for PS and higher for the other traits, thus indicating the prevalence of negative dominance effects for the former trait and of positive dominance effects for the others. Heterosis was <50% for SE, PS, and KW, ~50% for PH, ~160% for SW and NK, and even >200% for GY.

The F_2 performance was intermediate between parental mean and F_1 mean, with the exception of SE, for which F_2 showed the highest performance. This F_2 superiority was related to favorable maternal effects exerted by larger and healthier seed (harvested on the F_1 plants) in comparison with the seed of the other generations, harvested on the parental inbreds. Variation among mean values across parental lines, reciprocal F_1's, F_2, and BCs was largely accounted for by linear regression on the corresponding level of heterozygosity (Table 2); however, for SE, F_2 was not included in the regression analysis because of the bias previously discussed. Regression analysis provided information consistent with that obtained by comparing F_1 mean and parental mean, as the slope (b_1) was significant or highly significant for all traits except ASI and was positive for all traits except PS. The determination coefficient (R^2) was very close to 100%

TABLE 2

Mean values of the basic generations and parameters of the regression of the mean values on the corresponding level of heterozygosity

Generation	SE (%)	SW (g)	PS (days)	ASI (days)	PH (cm)	GY (Mg ha^{-1})	KW (mg)	NK (no.)
B73	67.6	2.19	78.9	2.0	174	3.81	265	229
H99	66.5[a], NS	2.41, NS	69.9**	2.1, NS	93**	2.48**	276, NS	143**
Mean	67.0	2.30	74.4	2.1	134	3.15	271	186
F_1	74.4*[,b]	5.8**	71.0**	1.4, NS	204**	10.69**	346**	492**
Heterosis (%)[c]	11	156	−5	−34	52	239	28	165
F_2	75.8	4.12	72.5	2.0	177	7.29	330	351
BC(B)	68.4	4.26	73.4	2.2	205	7.90	339	370
BC(H)	72.4	3.21	70.6	1.3	152	6.37	315	321
Mean (BC)	70.4	3.73	72.0	1.7	179	7.14	327	346
				Regression[d]				
b_0	66.9[e]	2.22	74.2	2.2	138	3.29	281	191
b_1	7.3*	3.59**	−3.4**	−0.7, NS	70**	7.55**	75**	306**
R^2 (%)	99.8	98.4	94.6	76.5	96.7	99.7	87.1	99.8
b_1/b_0 (%)	11	162	−5	−33	51	229	27	161

*$P \leq 0.05$, **$P \leq 0.01$. NS, not significant.

[a] Comparison between B73 and H99.

[b] Comparison between the mean of the two parents and the mean of the two reciprocal F_1's.

[c] Heterosis: $100 \times (F_1 - MP)/MP$, where MP is the midparent value.

[d] Linear regression of the mean value of the two parents, the mean value of the two reciprocal F_1's, the F_2, and the mean value of the two BCs on their heterozygosity level (*i.e.*, 0, 1, 0.5, and 0.5, respectively).

[e] The mean value of the F_2 was not considered in the regression analysis of SE.

for SE, GY, and NK, whereas for other traits (especially ASI and KW), the R^2 value was lower. For each trait, the percentage of b_1/b_0 corroborated the percentage of heterosis previously seen, as the two values were always close to one another.

RIL and TTC populations: ANOVA (not shown) revealed that the population × environment interaction was significant for most traits and at least partly due to magnitude effects, as indicated by the fact that the difference between the mean of the three TC populations and the mean of the RILs was larger in the environments with an overall higher mean. With respect to the analysis conducted within each population, $g \times e$ interaction was highly significant for GY and its components in all populations, whereas for SE and ASI it was significant only in the RIL population. Moreover, $g \times e$ interaction was always of greater importance for the RIL population; *e.g.*, for GY, the F ratio was 2.53 for the RILs and 1.50–1.74 for the TTC populations, consistently with the lower stability across environments expected for the homozygous materials. Despite the significance of $g \times e$ interaction, differences among genotypes across environments within each population were highly significant in all instances.

Table 3 shows mean values of the four populations across environments. The RIL population exhibited, as expected, the highest mean for PS and ASI and the lowest mean value for all other traits. The TC(B) mean was significantly higher than the TC(H) mean for all traits, thus confirming the prevalence of alleles with increasing effects provided by B73. The TC(F) mean was significantly different from the mean of the other two TC mean values for SW, ASI, KW, and NK.

RIL mean value (Table 3) and parental inbred mean value (Table 2) did not significantly differ for any trait, which is consistent with the care exerted to avoid any selection during the inbreeding process. The comparison between the best-performing RIL (Table 3) and the F_1 (Table 2), made according to Scheffe's test (not shown), revealed that the F_1 was exceeded, although not significantly, by the best RIL for SE and for low PS and significantly (at $P \leq 0.05$) for low ASI and for KW; these findings are consistent with the negligible or mild heterosis exhibited by such traits. On the other hand, the best RIL was significantly exceeded by F_1 for SW, PH, GY, and NK, which is consistent, too, with the more marked heterosis exhibited by these latter traits.

NCIII and TTC analyses: NCIII analysis led to the estimates of V_A, which were always significant at $P \leq 0.01$, and of V_D, always significant at $P \leq 0.01$, except for ASI, which was significant at $P \leq 0.05$ (Table 4). These significant estimates can be accounted for by considering that B73 and H99 belong to different heterotic groups and hence carry different alleles at many loci. The highest average degrees of dominance, all >1, were obtained for SW, GY, and NK, *i.e.*, for the three traits that showed the highest heterosis (as percentages, Table 2); on the other hand, the lowest average degree of dominance estimates were obtained for PS and ASI, which were among the traits with the lowest heterosis. Therefore, there was a clear relationship between the average degree of dominance calculated according to the NCIII

TABLE 3

Mean values of the RIL and TC populations

Population	SE (%)	SW (g)	PS (days)	ASI (days)	PH (cm)	KM[a] (%)	GY (Mg ha^{-1})	KW (mg)	NK (no.)
RIL	62.7	2.09	74.1	2.1	132	—	3.66	271	215
max	78.1	3.23	80.9	11.5	176	—	6.59	424	247
min	46.5	0.68	69.0	−1.3	87	—	1.34	208	102
TC(B)	71.5	4.19	74.0	2.0	206	28.8	7.50	321	371
TC(H)	68.0**[b]	3.47**	70.7**	1.2**	147**	26.5**	6.33**	307**	328**
Mean	69.7	3.83	72.4	1.6	177	27.7	6.92	314	350
TC(F)	69.2[c], NS	4.28**	72.3, NS	1.9**	177, NS	27.5, NS	6.90, NS	323**	340**

**$P \leq 0.01$. NS, not significant.

[a] KM not detected for RILs.

[b] Comparison between TC(B) and TC(H).

[c] Comparison between TC(F) and the mean of TC(B) and TC(H).

analysis and the level of heterosis calculated on the basic generations.

TTC analysis allowed a test for nonallelic interactions and significant additive × additive ([*aa*]) epistasis was detected for SW, ASI, KW, and NK. The significance of [*aa*] epistasis for these four traits can also be appreciated by comparing the TC(F) population mean value and the mean of the TC(B) and TC(H) populations (Table 3). The epistasis due to additive × dominance and dominance × dominance ([*ad*] and [*dd*]) was significant only for SE. For the traits that show significant epistasis, V_A and V_D estimates are to some extent biased (Kearsey and Pooni 1996) and so are the average degree of dominance estimates.

Main-effect QTL: QTL detected on RIL, TC(F), SUM, TC(B) H_{mp}, TC(H) H_{mp}, and the DIFF data set are reported in Table 5. The LOD threshold adopted, determined by permutations, ranged between 4.2 and 5.4 for $P = 0.10$ according to the trait, data set, and model (data not shown). A LOD threshold corresponding to $P = 0.25$ (ranging between 2.8 and 3.5) was adopted for "suggesting" the presence of a QTL when the LOD reached the threshold and its peak mapped approximately at the same position in more than one population/data set. As an example, the QTL controlling PH in bin 3.06 was indicated as suggested on the basis of its *a* effect, which reached the $P = 0.25$ threshold independently in RIL, TC(F), and the SUM data set. The effects of allele substitution were obtained from the contrasts as indicated in Table 1.

For SE, six QTL were found—one in the RILs, three in TC(F), and two in SUM data sets—whereas no QTL were found in TC(B) H_{mp}, TC(H) H_{mp}, and DIFF data sets, thus indicating the lack of QTL with appreciable dominance effects. Alleles increasing the trait were contributed by both H99 (bin 2.08, bin 2.09, bin 3.08, and bin 5.02) and by B73 (bin 4.07 and bin 6.07).

Twelve QTL were detected for SW. All the alleles increasing this trait were provided by B73, except for the QTL on bin 3.04. QTL found in the DIFF data set accounted for 31.3% of variation due to dominance effects, whereas only 13.6% of variation due to additive effects was accounted for by QTL found in the SUM data set.

Ten QTL were detected for PS. Most of them showed *a* effects, whereas only a few showed *d* effects, thus confirming that this trait is mostly controlled by genes with additive effects. Only 2 QTL detected in the DIFF data set were classified as overdominant. Two QTL with large additive effect were found in chromosome 8: one near *bnlg669* (bin 8.03) and the other near *phi121* (bin 8.05). This latter QTL colocates with *vegetative to generative transition 1* (*vgt1*), which is known to affect PS (Vladutu *et al.* 1999). A QTL for flowering date in this position (bin 8.05) was also found by Koester *et al.* (1993). Most of the plus alleles (determining lateness) were

TABLE 4

NCIII and TTC analyses

Parameter[a]	SE	SW	PS	ASI	PH	KM	GY	KW	NK
V_A[b]	74.0	0.20	1.94	0.39	200	3.34	1.02	676	2136
V_D[b]	24.9	0.13	0.14	0.03	37	0.32	0.71	82	1325
a.d.d.	0.82	1.11	0.38	0.40	0.61	0.44	1.18	0.49	1.11
[*aa*]	NS	**	NS	**	NS	NS	NS	**	**
[*ad*], [*dd*]	**	NS	NS	NS	NS	NS	NS	NS	NS

*$P \leq 0.05$, **$P \leq 0.01$. NS, not significant.

[a] Estimates of additive (V_A) and dominance (V_D) variance, average degree of dominance (a.d.d.), and tests for additive × additive ([*aa*]) and additive × dominance and dominance × dominance ([*aa*], [*dd*]) epistasis.

[b] V_A was highly significant ($P \leq 0.01$) for all traits; V_D was highly significant for all traits, except ASI ($P \leq 0.05$).

TABLE 5

Main-effect QTL detected in RIL, TC(F), SUM, TC(B) H_{mp}, TC(H) H_{mp}, and DIFF data sets

		RIL		TC(F)		SUM		TC(B) H_{mp}		TC(H) H_{mp}		DIFF		
		Effect[a]	R^2	Effect	R^2	Effect	R^2	Effect	R^2	Effect	R^2	Effect	R^2	
Bin	Marker interval	*a*	(%)	*a*	(%)	*a*	(%)	*d*	(%)	*d*	(%)	*d*	(%)	Degree of dominance[b]
							SE (%)							
2.08	*bnlg198–dupssr25*	1.2[c]	2.4[c]											PD
2.09	*bnlg1520–csu64a*			2.8	3.6									PD
3.08	*umc1140–umc1813*					3.8	7.0							PD
4.07	*bnlg1621–dupssr34*					−2.6	4.2							A
5.02	*bnlg105–phi008a*			2.5	3.8									PD
6.07	*dupssr15–phi123*			−3.9	6.8									PD
R^2_{Adj}			1.0		8.3		7.5		0.0		0.0		0.0	
							SW (g)							
2.09	*csu64a–bnlg468b*	−0.15	8.8											PD
3.02	*bnlg1325–bnlg1647*			−0.26	3.3									A
3.04	*dupssr5–umc42b*			*0.27*	*4.9*	0.23	5.4							PD
3.08	*dupssr17–umc1140*							0.53	10.4	0.35	4.7	0.45	15.2	OD
4.10	*umc1101–bnlg589*	−0.14	10.1			−0.28	7.6			0.35	6.4	0.36	13.9	D
7.02	*bnlg657–umc1015*							0.39	8.1			*0.24*	*7.0*	OD
8.01	*umc1075–bnlg1194*					*−0.20*	*4.2*							A
8.02	*umc1304–phi119*									0.36	5.7			D
8.03	*bnlg669–umc1904*							0.32	5.8			0.29	9.9	D
8.05	*phi121–bnlg666*					−0.21	4.9							A
9.05	*umc95–umc1231*							0.35	6.8					OD
10.06	*umc2003–bnl10.13*											0.25	6.9	OD
R^2_{Adj}			14.5		3.7		13.6		19.7		10.4		31.3	
							PS (days)							
1.07	*bnlg1025–dupssr12*	−0.59	7.8											PD
2.02	*bnlg1092–bnlg125*											−0.43	7.7	OD
2.08	*bnlg198–dupssr25*	0.09	1.2											PD
2.09	*bnlg1893–Z09989*	*0.82*	*14.5*	*0.43*	*5.9*	0.64	13.1							A
4.08	*dupssr28–phi093*							−0.31	1.6			−0.23	3.1	OD
7.02	*bnlg2203–phi008b*	0.88	13.5	0.64	9.5	0.81	16.2	−0.57	4.1			−0.43	7.6	PD
8.03	*bnlg669–umc1904*	−0.81	11.8	−0.46	4.9	−0.96	20.3							PD
8.05	*phi121–bnlg666*	−0.63	6.9	−0.91	16.5	−0.70	11.5							PD
9.05	*umc95–umc1231*			−0.36	4.1	−0.42	6.0							A
9.07	*umc1137–bnlg1129*	*−0.39*	*2.4*	−0.50	4.9	−0.23	1.1							PD
R^2_{Adj}			37.0		35.2		48.6		2.5		0.0		11.5	
							ASI (days)							
2.04	*dupssr27–bnlg381*											−0.15	2.9	D
2.09	*bnlg1520–csu64a*					*0.32*	*7.6*					*0.18*	*4.5*	PD

(*continued*)

TABLE 5

(Continued)

		RIL		TC(F)		SUM		TC(B) H_{mp}		TC(H) H_{mp}		DIFF		
		Effect[a]	R^2	Effect	R^2	Effect	R^2	Effect	R^2	Effect	R^2	Effect	R^2	
Bin	Marker interval	*a*	(%)	*a*	(%)	*a*	(%)	*d*	(%)	*d*	(%)	*d*	(%)	Degree of dominance[b]
3.01	*umc2071–bnlg1325*					0.38	6.0							A
3.02	*bnlg1647–phi029*					−0.41	9.9							PD
6.05	*mzePDKA–bnlg354*					0.34	9.6					0.15	3.7	PD
7.02	*phi008b–bnlg657*							−0.11	1.0					OD
7.05	*umc1295–php20690*			−0.32	3.4	−0.31	5.4							PD
8.03	*phi119–bnlg669*									0.45	8.3	*0.23*	*6.2*	OD
8.05	*bnlg666–bnlg162*									−0.40	6.7	*-0.22*	*6.0*	OD
9.07	*umc1137–bnlg1129*			−0.47	6.9									PD
R^2_{Adj}			0.0		6.5		20.8		1.0		8.3		10.4	
							PH (cm)							
1.03	*bnlg176–bnlg439*			6.9	6.1			4.5	2.9					PD
1.04	*bnlg2295–dupssr26*							4.3	5.7					D
1.07	*bnlg1025–dupssr12*	−5.2	11.1			−7.5	16.7			3.7	5.8			A
1.10	*bnlg1347–bnlg2331*							4.2	4.4			4.5	14.6	D
1.10	*bnlg2331–bnlg504*	−2.3	2.4			*−0.3*	*2.0*							PD
2.04	*dupssr27–bnlg381*											4.0	15.8	OD
2.05	*phi083–umc1028*	−2.9	3.3			−5.5	10.2			4.7	9.7			PD
2.08	*mmc0271–php20017*	−3.6	4.3	−3.7	3.5									A
2.09	*bnlg469b–bnlg1893*			−3.7	4.1									A
3.05	*XO6755–dupssr23*									6.1	13.8	5.0	22.7	OD
3.06	*dupssr23–bnlg197*	*−4.7*	*5.6*	*−4.9*	*4.3*	*-4.1*	*3.5*							A
4.03	*nc135–umc2176*							4.3	4.9					OD
4.04	*umc1963–nc005*	−3.2	4.2											A
4.07	*bnlg1621–dupssr34*											3.5	9.5	D
4.10	*bnlg589–bnlg1917*							3.0	3.1			3.0	9.8	OD
6.07	*phi070–dupssr15*							4.8	7.5			1.3	2.3	OD
7.03	*umc1134–umc1029*							4.3	3.9			3.1	8.2	OD
7.04	*bnl7.61–umc1295*					5.1	8.9							PD
8.01	*umc1075–bnlg1194*	−5.9	9.3											PD
8.03	*bnlg669–umc1904*									2.7	2.8	3.0	8.3	D
8.05	*bnlg666–bnlg162*	−5.6	10.3	−5.7	7.4	−7.9	15.3			4.9	5.0	3.9	10.9	PD
8.07	*umc1268–npi438b*	−4.4	8.4	*−4.4*	*5.6*	−5.5	9.8							PD
10.03	*bnlg1451–umc2016*											4.1	8.4	OD
10.04	*umc64a–umc2003*									5.5	6.7	2.4	3.3	D
R^2_{Adj}			34.0		22.5		37.7		17.4		33.9		57.0	
							KM (%)							
2.08	*bnlg198–dupssr25*		—			1.01	7.8		—		—			PD
2.09	*csu64a–bnlg469b*		—	0.49	2.2				—		—			PD

(*continued*)

TABLE 5

(Continued)

		RIL		TC(F)		SUM		TC(B) H_{mp}		TC(H) H_{mp}		DIFF		
		Effect[a]	R^2	Effect	R^2	Effect	R^2	Effect	R^2	Effect	R^2	Effect	R^2	
Bin	Marker interval	*a*	(%)	*a*	(%)	*a*	(%)	*d*	(%)	*d*	(%)	*d*	(%)	Degree of dominance[b]
2.09	*Z09989–bnl17.14*		—	0.38	1.8	*0.43*	*13.6*		—		—			A
5.03	*dupssr7–umc1482*		—	−0.82	11.9				—		—			A
5.08	*umc1792–bnlg386*		—	0.57	4.7				—		—			A
8.03	*bnlg669–umc1904*		—			−0.82	4.3		—		—			PD
8.05	*bnlg666–bnlg162*		—			−0.38	1.1		—		—			PD
9.03	*phi065–bnlg430*		—			−0.75	4.4		—		—			PD
9.05	*umc1231–bnlg1525*		—	−0.41	3.4				—		—			A
9.07	*umc1137–bnlg1129*		—	−0.64	5.9	−1.85	16.4		—		—			A
R^2_{Adj}			—		26.8		28.3		—		—		0.0	
							GY (Mg ha^{-1})							
1.04	*bnlg2295–dupssr26*							0.15	2.7					OD
1.06	*dupssr26–umc1035*							0.35	3.1			0.25	2.9	OD
1.07	*bnlg1556–bnlg1025*									0.58	8.1	0.37	5.0	OD
2.04	*dupssr27–bnlg381*							0.55	7.9					OD
2.07	*phi127–mmc0271*					0.23	2.3	0.34	3.1			0.40	9.3	OD
3.02	*bnlg1647–phi029*	0.24	5.5	0.54	12.2	0.35	6.2							A
3.05	*XO6755–dupssr23*							0.35	3.4			0.46	9.8	OD
4.03	*nc135–umc2176*									1.26	13.1	0.81	15.4	OD
4.07	*dupssr34–dupssr28*									0.58	7.3			OD
4.10	*umc1011–bnlg589*							0.47	7.4			0.44	11.1	OD
5.04	*dupssr10–dupssr7*			−0.53	11.7	−0.33	5.1			0.61	11.2			D
6.01	*bnlg161b–bnlg1371*	0.42	15.9			0.33	5.7	0.62	12.2	0.50	9.3	0.53	18.2	OD
6.07	*phi070–dupssr15*							0.52	8.8			0.11	0.8	OD
7.04	*umc1029–bnl7.61*					0.37	6.8							A
8.03	*umc1904–bnl9.08*							0.63	9.0			0.32	4.3	OD
8.05	*phi121–bnlg666*	−0.32	7.2	−0.84	19.6	−0.77	18.5			0.92	17.9	0.36	3.8	PD
9.02	*dupssr6–bnlg1401*									0.45	5.4			OD
9.05	*bnlg1209–umc95*							0.67	10.5			0.60	14.3	OD
9.07	*umc1137–bnlg1129*					−0.31	4.4							PD
10.03	*bnlg1451–umc2016*							0.58	7.0	0.55	4.6	0.85	16.6	OD
10.04	*umc64a–umc2003*									0.66	8.7	0.17	1.1	OD
R^2_{Adj}			19.9		30.4		29.2		40.1		48.6		53.7	
							KW (mg)							
2.02	*bnlg1092–bnlg125*											5.8	4.0	D
2.05	*umc1028–phi127*											10.8	16.1	OD
2.08	*bnlg198–dupssr25*					*7.4*	*5.1*							A
3.01	*umc2071–bnlg1325*	−5.2	3.0											PD

(*continued*)

TABLE 5

(Continued)

		RIL		TC(F)		SUM		TC(B) H_{mp}		TC(H) H_{mp}		DIFF		
		Effect[a]	R^2	Effect	R^2	Effect	R^2	Effect	R^2	Effect	R^2	Effect	R^2	
Bin	Marker interval	*a*	(%)	*a*	(%)	*a*	(%)	*d*	(%)	*d*	(%)	*d*	(%)	Degree of dominance[b]
4.08	*dupssr34–dupssr28*									7.9	4.3	5.9	6.5	D
4.08	*phi093–umc1101*	12.5	7.3			14.2	8.8							A
5.03	*phi008a–bnlg557*	−9.3	2.9	−12.5	12.9	−10.0	4.9							A
5.03	*dupssr7–umc1482*	−7.6	4.0			−7.3	3.5			11.5	11.5	6.9	11.6	D
7.03	*bnlg434–umc1134*									9.5	7.5	7.5	12.3	OD
7.04	*umc1029–bnl7.61*			11.4	12.7									A
8.03	*bnlg669–umc1904*			−8.4	5.8	−7.0	3.6							A
8.05	*phi121–bnlg666*			−10.6	8.1	−10.1	6.6							A
9.01	*umc1040–php10005*											1.8	0.9	OD
R^2_{Adj}			10.4		33.4		32.9		0.0		17.5		31.7	
							NK (no.)							
1.06	*umc1035–bnlg1556*									36	9.7	19	7.4	OD
1.07	*bnlg1025–dupssr12*									13	2.1	9	2.2	OD
2.07	*phi127–mmc0271*							21	4.3			14	6.9	D
3.05	*XO6755–dupssr23*									35	12.7	23	13.2	OD
4.03	*nc135–umc2176*									53	15.1	16	8.2	D
4.10	*umc1101–bnlg589*									27	10.0	22	15.2	OD
6.01	*bnlg161b–bnlg1371*	18	6.6			21	6.2							PD
6.01	*bnlg1371–bnlg391*											23	15.3	OD
6.04	*dupssr18–umc1014*	42	5.5											A
6.07	*phi070–dupssr15*							23	5.9					OD
8.02	*bnlg1194–umc1304*					−19	6.1			20	4.3			D
8.03	*bnlg669–umc1904*							30	9.6			18	8.4	OD
8.03	*bnl9.08–phi121*	−19	8.1							20	4.5	14	3.7	D
9.03	*bnlg430–bnlg1209*											20	5.1	OD
9.05	*bnlg1209–umc95*							38	12.0			8	1.0	OD
10.03	*bnlg1451–umc2016*							40	10.7	38	9.8	39	20.8	OD
10.04	*umc64a–umc2003*									36	17.1	7	1.3	D
R^2_{Adj}			20.5		0.0		9.6		27.4		40.8		61.6	

[a] The effects are obtained from the contrasts as indicated in Table 1. Effects obtained in TC(B) H_{mp}, TC(H) H_{mp}, and TC(F) were multiplied by two, and the values obtained in TC(H) H_{mp} and the DIFF data set were also multiplied by (−1).

[b] The degree of dominance for all QTL declared as significant in any data set was determined after estimating their additive and dominance effects, respectively, in SUM and DIFF data sets. QTL were classified according to their $|d/a|$ ratio as additive (A; $|d/a| < 0.2$), partially dominant (PD; $0.2 \leq |d/a| < 0.8$), dominant (D; $0.8 \leq |d/a| < 1.2$), and overdominant (OD; $|d/a| \geq 1.2$) (Stuber *et al.* 1987).

[c] Effect and R^2 of a "significant QTL," *i.e.*, a QTL with LOD reaching the threshold for $P \leq 0.10$ determined by 1000 permutations (the threshold corresponded to a minimum value of LOD = 4.2). Entries in italics indicate the effect and R^2 of a "suggested QTL," *i.e.*, a QTL with LOD reaching the threshold for $P \leq 0.25$ (minimum LOD = 2.8) and mapping approximately at the same position in more than one population/data set or colocating with a significant QTL controlling another trait.

contributed by B73, as revealed by the prevalence of the negative sign of the *a* effects. Moreover, *d* effects were always negative, indicating, as expected, dominance for earliness.

Ten QTL were found for ASI. Appreciable dominance effects were detected for 6 QTL; dominance was negative for 3 of them and positive for the other 3. This finding is noteworthy because it indicates that the lack of significant heterosis observed for ASI in the basic generations is at least partly due to the presence of QTL with counterbalancing dominance effects, rather than to the presence of QTL with additive effects only.

Twenty-four QTL were found for PH. This high number can be accounted for by considering the large difference between the two parental inbreds for this trait. Significant additive effects were shown by 12 QTL, most of which (*i.e.*, 10) with a negative sign, thus indicating that the plus allele was more often provided, as expected, by the taller B73 parent. The two exceptions with positive sign were found in bins 1.03 and 7.04. Two important QTL with additive effect were found in bins 1.07 and 2.05, *i.e.*, in regions where Lubberstedt *et al.* (1997) and Melchinger *et al.* (1998) also found QTL for PH. Large dominant effects were found for a QTL on bin 3.05 (accounting for the 22.7% of the variance due to dominance). On the basis of the d/a ratio, 7 QTL showed overdominance, 12 showed partial-to-complete dominance, and the other 5 showed absence of dominance.

Kernel moisture was not measured in RILs; thus TC(B) H_{mp} and TC(H) H_{mp} were not calculated. Ten QTL were found by analyzing TC(F) and the SUM data set, whereas no QTL was found in the DIFF data set. These results indicate that, for the identified QTL controlling KM, additive effects are important and dominance effects are negligible. For most QTL, the plus allele was provided by B73, consistently with its greater lateness at flowering.

For GY we identified 21 QTL, 16 of which showed overdominance. Three QTL with important additive effects were found in bins 3.02, 7.04, and 8.05; this latter QTL displayed the largest R^2 value in both TC(F) and SUM data set, with the increasing allele provided by B73. This QTL also displayed a significant dominance effect, evidenced particularly in TC(H) H_{mp}. Beavis *et al.* (1994) and Melchinger *et al.* (1998) also reported the presence of important QTL for GY in this region. The QTL with the largest dominance effects were detected on chromosome 4 (close to *nc135*), on chromosome 9 (*bnlg1209*), and on chromosome 10 (*bnlg1451*). The QTL on bin 10.03 displayed a dominance effect of 0.85 Mg ha^{-1}, with a LOD score of 16.79 and a partial R^2 of 16.6. Other QTL with important *d* effects were located in bins 3.05 and 4.10.

Thirteen QTL were found for KW. Seven QTL showed important additive effect and 5 of them were previously reported in the literature. A QTL located on bin 2.08 was found by Goldman *et al.* (1994) and Austin and Lee (1996), whereas a QTL on chromosome 4 near *umc1101* was reported by Veldboom and Lee (1996). The QTL on bin 5.03, near *phi008a*, was reported by Abler *et al.* (1991) while the QTL on bins 8.03 and 8.05 were reported by Schon *et al.* (1994) and Bohn *et al.* (1996), respectively. The QTL on chromosome 5, near *dupssr7*, displayed both additive and dominance effects.

Seventeen QTL were detected for NK. Most of them (*i.e.*, 13) were detected in the DIFF data set and accounted for 61.6% of the dominance variation. The $|d/a|$ ratio was for most QTL in the dominance–overdominance range, similar to what was observed for GY. By far, the most important dominance effect was displayed by the QTL on bin 10.03, as was noted for GY. An important QTL for NK was also found in this region by Ribaut *et al.* (1997) under a water stress regime in tropical maize. A notable dominance effect was also revealed by QTL on bins 3.05, 4.10, and 6.01.

In principle, all data sets identifying QTL with additive and/or dominance effects should locate the same QTL. Therefore, to obtain more complete information, all QTL detected in at least one data set were also estimated in all the other data sets, regardless of statistical significance. All additive and dominance effects are reported in supplemental Table 1 (at http://www.genetics.org/supplemental/). Even though some estimates could be biased, especially for nonsignificant QTL, it is interesting to note that the information contributed by the different data sets is generally consistent.

Our populations share B73 as a parental line with the populations investigated by Stuber *et al.* (1992) and Cockerham and Zeng (1996), who also identified QTL with important overdominance effects. A comparison was made between genomic regions showing high values of overdominance in this study and in Cockerham and Zeng (1996) on corresponding traits. For this purpose, the degree of dominance ($|d/a|$) at each marker was calculated from the *d* and *a* estimates reported in Cockerham and Zeng (1996) (supplemental Table 2 at http://www.genetics.org/supplemental/). Of the 16 overdominant QTL for GY that we found in the B73 × H99 background, 5 colocated with QTL showing $|d/a|$ higher than the average for the trait identified in the B73 × Mo17-derived material (*i.e.*, those in bins 1.06, 1.07, 2.04, 9.05, and 10.04). Eight additional QTL showing overdominance in this work (*e.g.*, the QTL in bin 1.04) were identified in bins adjacent to the ones identified by Cockerham and Zeng (1996).

Epistatic QTL: Most of the main-effect QTL detected with PLABQTL for SUM and DIFF data sets were confirmed in the analysis performed with QTLMapper when epistasis was not included in the model (data not shown) and the QTL not in common were those with low R^2. However, the analysis performed by comparing different CIM models should permit a better resolution than the one utilized in QTLMapper (Zeng 1994) and thus it is expected to also reveal minor factors. In addition, the high thresholds chosen for the QTLMapper analysis

should have limited the occurrence of false positives (Wang *et al.* 1999). When epistasis was included in the model, no additional main-effect QTL were found and almost all QTL were located on the same or on the adjacent interval as with the model without epistasis (data not shown). Table 6 shows digenic epistatic interactions detected in SUM and DIFF data sets. Epistatic QTL were detected for all traits, with a minimum number for PH (two interactions in the SUM data set) and a maximum of eight for SW (five in SUM and three in DIFF). Most of the detected interactions involved QTL with nonsignificant main effects and each interaction generally showed modest R^2 for all traits. To validate such interactions, phenotypic data were sorted into genotypic classes by using markers flanking the interacting genomic regions, and a linear model was used to test the significance of the difference among classes and of the interactions. The results (not shown) substantially confirmed the findings obtained with QTLMapper. The proportion of total variation explained by all epistatic interactions, either in the SUM or the DIFF data set, was <20% in most cases. The highest values of total R^2 were observed for SW in the SUM data set (34.8%) and for SE in the DIFF data set (29.8%), which mainly reflect the additive × additive and dominance × dominance epistatic interactions, respectively. These findings are consistent with the ones previously seen in TTC genetic analysis, since significant [*aa*] epistasis was detected for SW and significant [*dd*] epistasis was detected for SE. Other important total R^2 in SUM data sets were observed for ASI (25.7%) and NK (21.7%) and in both cases [*aa*] epistasis was also found with the classical TTC analysis. On the other hand, epistasis was of much lesser importance for PH ($R^2 = 11.3\%$), a result that is consistent, too, with the negligible epistasis detected by TTC analysis.

Relationship between molecular marker heterozygosity and phenotypic performance: The highest correlation coefficients (Table 7) between level of heterozygosity and performance *per se* of the two TC populations were shown for GY [$r = 0.61$** in TC(B) and $r = 0.54$** in TC(H)]. Significant coefficients were also shown for those traits that, together with GY, had revealed the highest dominance ratios, such as SW and NK. On the other hand, correlation coefficients were not significant for those traits showing a low degree of dominance, especially SE, PS, and ASI. For each trait, the correlation coefficient calculated within the TC(B) population did not significantly differ from the coefficient calculated in TC(H); the only exception was for PH for which the coefficient in TC(H) ($r = 0.49$) was significantly higher ($P \leq 0.01$) than that in TC(B) ($r = 0.17$).

To have a better insight into the relationships between phenotypic performance and heterozygosity, the results of regression analysis of the former on the latter are shown for two representative traits, PH and GY (Figure 1). For PH, the slope of the performance *per se* in the TC(B) was significantly lower than the slope in the TC(H), while for GY, the slopes in the two TC populations were not statistically different.

The analysis of the relationship between level of heterozygosity and of heterosis [as evaluated in TC(B) H_{mp} and TC(H) H_{mp}] provided correlation coefficients that, for several traits, were slightly higher than those previously seen. The highest correlation coefficients were again found for GY [$r = 0.68$** in TC(B) H_{mp} and $r = 0.58$** in TC(H) H_{mp}].

Using the DIFF data set as the dependent variable, correlation coefficients in most cases were higher (as absolute values) than those obtained with the two previous approaches. Such increases were more notable for PH, GY, and its components. GY confirmed the highest correlation ($r = 0.76$**), indicating that 58% of the variation observed for the DIFF data set could be accounted for by linear relationship with the heterozygosity level [referred to the TC(H) hybrids].

DISCUSSION

Heterosis for the investigated traits: Heterosis proved to be of some importance for PH and rather sizable for SW, NK, and especially GY. Heterosis for SW is noteworthy since it indicates that differences in plant vigor among the investigated materials are already well established in early growth stages, consistent with the findings of previous studies conducted on maize (Tollenaar *et al.* 2004; Hoecker *et al.* 2006) as well as other species (see Meyer *et al.* 2004 for a review). The highest heterosis was exhibited by GY, with a value (>200%) close to the highest values reported in literature for crosses between inbred lines (see Hallauer and Miranda 1988). When heterosis is >100%, as for the above three traits, complete dominance alone might not be adequate to account for heterosis, and the contribution of overdominance and/or epistasis should be also considered (Wricke and Weber 1986). As to GY, however, the high heterosis observed in our study can be at least partly related to the modest yield level achieved by the two parental inbreds (on average 3.15 Mg ha^{-1}). All materials were grown at the same plant density (*i.e.*, 6.25 plants m^{-2}), which, also given the overall favorable growing conditions achieved in the trials, was likely more suitable for the hybrid than for the two inbreds (especially for the small-sized H99). According to Sprague (1983) and Duvick (2005), heterosis should be studied by growing the parental inbreds and hybrids at their optimum plant density, thus allowing the achievement of comparable leaf-area indexes.

Allelic interactions (dominance *vs.* overdominance): *Classical genetic analysis:* For SW, NK, and GY, the average degree of dominance proved to be >1, thus suggesting an important contribution of overdominance to the sizable heterosis of these traits. Bingham (1998) summarized the results of studies in which F_2 maize populations were randomly mated for six (or even more)

TABLE 6

Analysis of epistatic QTL detected in SUM and DIFF data sets

Trait and data set	Bin	Marker interval	Bin	Marker interval	LOD	a_i[a]	a_j[a]	aa_{ij}[a]	R^2 (%)[b]	R^2 (%)[c]
				SE(%)						
SUM	4.08	*phi093–umc1101*	4.10	*bnlg1917–umc1058*	3.5			4.2	11.4	11.4
DIFF	1.09	*phi011–bnlg1720*	7.03	*bnlg434–umc1134*	5.0			−3.0	7.1	
	2.09	*bnlg469b–bnlg1893*	5.03	*phi008a–bnlg557*	4.7			2.6	8.0	
	5.06	*bnlg609–phi085*	10.07	*umc1196–bnlg1360*	4.1			2.4	9.4	
	7.01	*php20581–umc1986*	10.01	*php20075–bnlg1451*	3.3			2.7	5.3	29.8
				SW (g)						
SUM	3.09	*bnlg1754–dupssr8*	8.06	*bnlg240–umc1149*	4.8			0.32	6.1	
	4.03	*nc135–umc2176*	8.03	*bnlg669–umc1904*	4.1			0.31	8.1	
	4.05	*nc005–umc1511*	8.03	*bnlg669–umc1904*	4.4			−0.33	7.6	
	4.10	*bnlg1917–umc1058*	7.04	*bnl7.61–umc1295*	4.3			−0.30	5.7	
	6.01	*bnlg161b–bnlg1371*	8.05	*bnlg666–bnlg162*	4.3			−0.34	7.3	34.8
DIFF	1.07	*bnlg1025–dupssr12*	6.07	*dupssr15–phi123*	4.0			0.25	5.9	
	1.10	*bnlg1347–bnlg2331*	5.02	*phi024–umc1587*	4.6			0.30	5.9	
	2.08	*bnlg198–dupssr25*	7.03	*bnlg434–umc1134*	4.6			0.31	5.4	17.1
				PS (days)						
DIFF	1.04	*phi001–bnlg1016*	5.05	*umc1482–phi087*	3.5			0.37	5.9	
	2.02	*bnlg1092–bnlg125*	7.02	*bnlg2203–phi008b*	7.0	−0.41**	−0.35**	−0.38	5.5	
	3.08	*umc1140–umc1813*	4.08	*phi093–umc1101*	3.5			0.46	6.8	
	7.05	*php20690–phi082*	8.05	*phi121–bnlg666*	5.9			0.55	8.1	26.2
				ASI (days)						
SUM	2.09	*bnlg1520–csu64a*	4.07	*bnlg1621–dupssr34*	6.5	0.42**		−0.42	11.4	
	3.08	*dupssr17–umc1140*	7.02	*umc1986–phi034*	5.0			0.40	5.9	
	4.03	*nc135–umc2176*	7.02	*umc1986–phi034*	5.0			0.36	8.4	25.7
				PH (cm)						
SUM	1.08	*dupssr12–phi011*	4.10	*bnlg589–bnlg1917*	9.2	−7.2**		5.5	5.9	
	1.10	*bnlg2331–bnlg504*	9.03	*phi065–bnlg430*	3.9			5.9	5.4	11.3
				KM (%)						
SUM	1.03	*bnlg176–bnlg439*	7.05	*umc1295–php20690*	4.7			0.63	6.5	
	6.07	*dupssr15–phi123*	10.03	*bnlg1451–umc2016*	3.5			0.61	6.4	12.8
DIFF	2.02	*bnlg1092–bnlg125*	2.07	*phi127–mmc0271*	4.8			−0.58	5.0	
	6.04	*umc1014–mzetra*	7.02	*umc1986–phi034*	5.6			0.64	7.6	12.6

(*continued*)

TABLE 6

(Continued)

Trait and data set	Bin	Marker interval	Bin	Marker interval	LOD	a_i[a]	a_j[a]	aa_{ij}[a]	R^2 (%)[b]	R^2 (%)[c]
				GY (Mg ha^{-1})						
SUM	1.07	*bnlg1025–dupssr12*	8.05	*bnlg162–bnlg240*	5.6			0.46	5.7	
	3.02	*bnlg1647–phi029*	4.04	*umc1963–nc005*	8.4		0.30**	−0.57	7.1	
	3.04	*umc42b–bnlg602*	9.05	*umc95–umc1231*	6.4			0.48	5.1	17.9
DIFF	3.04	*bnlg1019–dupssr5*	8.05	*phi121–bnlg666*	8.2		0.58**	−0.66	5.8	5.8
				KW (mg)						
SUM	2.08	*dupssr25–bnlg1520*	7.03	*umc1015–bnlg434*	7.8			10.3	5.9	
	4.07	*dupssr34–dupssr28*	7.02	*phi034–bnlg398*	5.1			−9.7	3.6	
	6.00	*phi075–bnlg161b*	8.03	*bnlg669–umc1904*	14.3		−14.1**	−9.9	6.8	16.3
DIFF	2.04	*bnlg381–phi083*	4.05	*nc005–umc1511*	4.8			−5.8	5.9	
	4.03	*nc135–umc2176*	8.03	*phi119–bnlg669*	5.8			−4.9	6.8	
	8.03	*phi119–bnlg669*	10.07	*bnlg1360–bnlg1185*	3.4			−5.5	6.0	18.7
				NK (no.)						
SUM	2.02	*bnlg1092–bnlg125*	3.04	*dupssr5–umc42b*	4.6			−24.8	6.8	
	3.09	*bnlg1754–dupssr8*	9.03	*phi065–bnlg430*	5.2			−26.0	6.5	
	6.01	*bnlg161b–bnlg1371*	8.02	*bnlg1194–umc1304*	7.0	13.3**	−19.7**	20.6	8.5	21.7

**$P \leq 0.01$.

[a] a_i and a_j are the main effects of the loci i and j, and aa_{ij} is the epistatic effect between loci i and j.

[b] Percentage of the total variation explained by the aa_{ij}.

[c] Percentage of the total variation explained by all the interactions detected for the trait.

TABLE 7

Correlation coefficients between molecular marker heterozygosity and phenotypic performance

	Performance *per se*		Heterosis		
Trait	TC(B)	TC(H)	TC(B) H_{mp}	TC(H) H_{mp}	DIFF
SE	0.05, NS	−0.15, NS	−0.01, NS	−0.11, NS	−0.10, NS
SW	0.33**	0.31**	0.37**	0.28**	0.44**
PS	−0.05, NS	−0.16, NS	−0.05, NS	−0.26**	−0.20*
ASI	−0.08, NS	0.01, NS	−0.03, NS	−0.04, NS	−0.07, NS
PH	0.17*	0.49**	0.47**	0.52**	0.64**
KM	0.24**	0.04, NS	[a]	[a]	0.31**
GY	0.61**	0.54**	0.68**	0.58**	0.76**
KW	0.19*	0.37**	0.31**	0.46**	0.57**
NK	0.58**	0.48**	0.61**	0.52**	0.72**

Correlation between heterozygosity level and the performance *per se* of each TC [TC(B) and TC(H)], the effect of heterosis in each TC [TC(B) H_{mp} and TC(H) H_{mp}], and the effect of heterosis over TCs (DIFF). *$P \leq 0.05$, **$P \leq 0.01$. NS, not significant.

[a] KM was not investigated in parental inbreds and RILs.

generations; he showed that the dominance degree declined from values >1.5 to values <1.0 after random mating. These results clearly indicate that pseudo-overdominance, rather than true overdominance, was the main cause of the high dominance degree in those original F_2 populations. On the other hand, Lu *et al.* (2003), after three generations of random mating in an F_2 source, found for GY a dominance degree still rather high (1.79), suggesting that three generations were not adequate to consistently reduce the original linkage disequilibrium (the linkage being very tight) and/or that true overdominance was important in that material. In our study, the source F_2 population was not subjected to random mating before starting the inbreeding process for producing the RILs; on the other hand, such an inbreeding process should have led to some recombination, the overall amount of which is expected to be similar to the amount of recombination obtained in an F_2 population after two generations of random mating (Darvasi and Soller 1995). Therefore, we may assume that the original linkage disequilibrium still persisted in our RIL population, at least for tightly linked genes, so that pseudo-overdominance might have played a role in determining the high average degree of dominance for SW, GY, and NK.

QTL analysis: Our analyses allowed the identification of several QTL affecting the variation of the investigated traits. When a QTL effect was detected in more than one analysis [*i.e.*, in RIL, TC(F), and the SUM data set for the additive effect and in TC(B) H_{mp}, TC(H) H_{mp}, and the DIFF data set for the dominance effect], the sign of such an effect was always the same in all cases. This consistency is interesting, especially for additive effects, because they are estimated in three sets of independent data: RIL, TC(F), and the SUM data set. In this connection, it should be noted that the detection of QTL in NCIII and TTC could potentially benefit from statistical methods combining the information from multiple

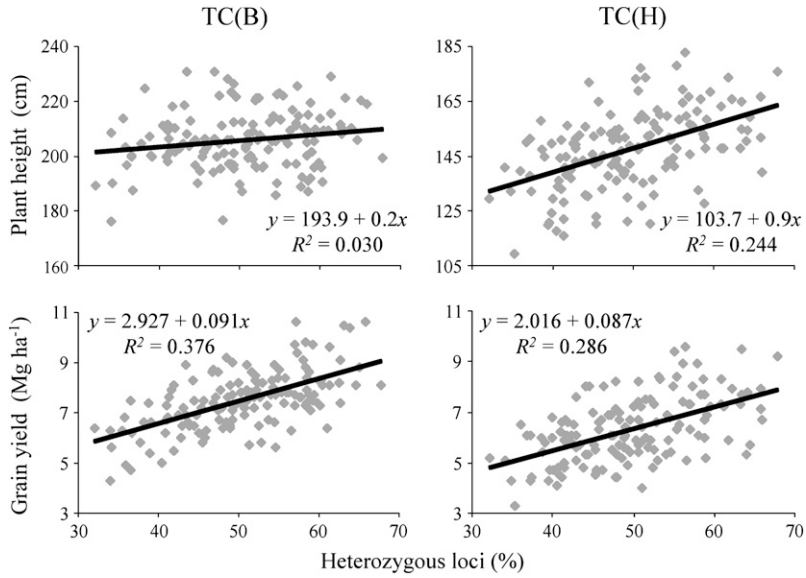

Figure 1.—Regression analysis of phenotypic performance *per se* for plant height and grain yield on percentage of heterozygous molecular marker loci in TC(B) and TC(H) populations.

populations. For this purpose, theories like the one originally proposed by Jiang and Zeng (1995), to investigate the same population for multiple traits and/or in multiple environments, should be further developed.

Dominance effects of the detected QTL were always unidirectional, with the only exception being ASI, *i.e.*, negative for PS and positive for all other traits. Moreover, for a given trait, the whole proportion of QTL with overdominance and dominance effects was consistent with the level of heterosis (as shown by the basic generations) and with the average degree of dominance (as provided by the NCIII analysis). In fact, traits with the highest proportion of QTL with overdominance or dominance effects, particularly GY and NK, were also the ones that had shown the highest level of heterosis and the highest degree of dominance. Accordingly, traits with the lowest proportion of QTL with overdominance or dominance effects, such as SE, PS, KM, and KW, were also the ones with the lowest level of heterosis and/or dominance degree. These findings indicate a strong consistency between information obtained by classical genetic analysis and QTL analysis. However, QTL exhibiting high overdominance effects are not necessarily indicative of true overdominance, but rather they can be the result of dominant alleles linked in repulsion (pseudo-overdominance). In their pioneer work, Stuber *et al.* (1992) detected several QTL for GY, most of which with overdominant gene action. Cockerham and Zeng (1996) reanalyzed the data of Stuber *et al.* (1992) with a different statistical approach and confirmed the importance of overdominance gene action for GY. Later, Graham *et al.* (1997), further investigating a major overdominant QTL identified in chromosome 5, dissected it into two QTL in repulsion-phase linkage, both acting in a dominant manner. Moreover, Stuber and co-workers (LeDeaux *et al.* 2006), in a further development of their study, identified substantially the same QTL for GY as in the earlier investigation, but evidenced mainly dominance rather than overdominance effects. These authors attributed such a discrepancy to the denser marker map used in the more recent study together with a different QTL analysis (CIM). On the other hand, in the Lu *et al.* (2003) study, 86% of QTL for GY showed overdominance even after two generations of intermating, leaving the possibility of true overdominance still open. Interestingly, overdominance associated with traits involved in reproductive fitness, such as total yield and number of seeds per plant, was also detected in a population of tomato introgression lines (Semel *et al.* 2006). As the authors pointed out, the association of overdominant QTL with the reproductive traits could represent an important evolutionary advantage and was likely favored during domestication.

Nonallelic interactions (epistasis): *Classical genetic analysis:* Epistasis appeared of lesser importance than intralocus interaction in affecting heterosis, as pointed out by regression analysis of agronomic performance on heterozygosity level as well as by TTC analysis. As to GY, the coefficient of determination of the regression was rather close to 100%, and in TTC analysis across environments neither [*aa*] nor [*ad*] and [*dd*] proved to be significant. On the other hand, for both yield components, *i.e.*, KW and NK, the [*aa*] epistatic component was significant. Wolf and Hallauer (1997), analyzing a TTC derived from the maize single cross B73 × Mo17, observed that epistasis across environments was more important for yield components than for yield itself. It should be stressed, however, that in our study, unlike that of Wolf and Hallauer (1997), entries were not divided into sets, being distributed according to a split-plot design. This might have led to less accurate estimates of the [*aa*] component since whole-plot error (used for testing its significance) was often larger than the subplot error (used to estimate the significance of the [*ad*] and [*dd*] component).

QTL analysis: Analysis of epistatic interactions among QTL should be considered with caution because of the limited size of the mapping population considered here (Gallais and Rives 1993) and for the possible detection of false positives due to the method adopted. Nevertheless, only a small number of interacting QTL were found, even for complex traits such as GY. This finding is in agreement with the modest role of epistasis detected with classical genetic analysis. In fact, even for those traits displaying the highest heterosis, *i.e.*, GY and NK, analysis of the DIFF data set revealed only one digenic interaction, thus suggesting that the dominance × dominance epistatic component was not important in determining heterosis in these two traits. Heterosis could derive from dominance × dominance epistasis (Goodnight 1999), but this does not seem to be the case in our materials. Edwards *et al.* (1987) conducted a QTL analysis in two different F_2 populations and found that very few QTL (<3%) were involved in epistatic interaction. A similar finding of lack of epistasis was obtained in the QTL analysis conducted by Stuber *et al.* (1992) on the materials arising from B73 × Mo17; however, in the subsequent analysis of Cockerham and Zeng (1996), significant epistasis between linked QTL was revealed. Melchinger *et al.* (1998) performed a QTL analysis on materials arising from the cross between two inbreds belonging to the same germplasm group and did not reveal any significant digenic interactions among the identified QTL. On the other hand, epistasis for complex traits appears to be more pronounced in self-pollinated crop species, such as rice (*Oryza sativa* L.; Yu *et al.* 1997). This is not surprising, since coadapted gene complexes exhibiting favorable epistatic effects can be more easily maintained in self-pollinated species than in cross-pollinated species (Allard 1988). Sizable epistatic effects were found by several studies (Li *et al.* 1997, 2001; Luo *et al.* 2001) in crosses among rice lines derived from different subspecies (*i.e.*, *indica* × *japonica*). These findings are corroborated by the study of Li *et al.* (1997)

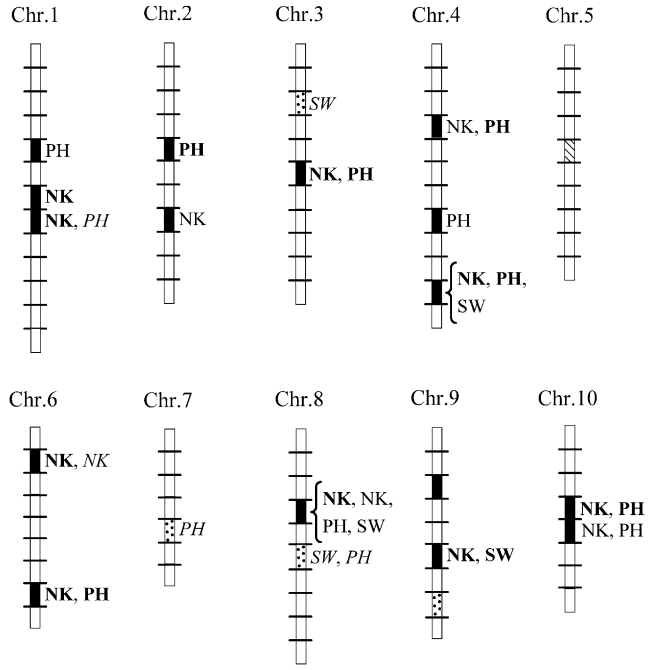

FIGURE 2.—Bin allocation of the 21 QTL for grain yield showing overdominant (solid segments), dominant (segments with stripes), and partially dominant or additive (dotted segments) effects. Segments along the chromosomes represent the bins. QTL detected for the other heterotic traits—SW, PH, and NK—and colocating with those for grain yield are indicated in boldface type if they are overdominant, roman type if dominant, and italic type if partially dominant or additive.

who noted that interactions among alleles from the same parent were often more favorable than the recombinant-type interactions involving alleles from the two parents.

Relationships between molecular marker heterozygosity and phenotypic performance: The highest relationships between heterozygosity level and phenotypic performance were obtained for those traits showing the highest levels of heterosis and of degree of dominance. The role played by dominance, together with the allele distribution between parental inbreds, was evidenced by the comparison between the slopes of the regression analysis conducted in both TC(B) and TC(H) populations for PH. The marked difference between the two trends can be at least partly accounted for by a prevalence of allele association in the two parents, with B73 carrying the dominant alleles determining taller plants but also exerting a masking effect on the variation of this trait. This hypothesis of allele association is consistent with the large difference found between the two parental inbred lines for the trait (as shown in Table 2). On the contrary, when GY was examined, the slopes of the two TC populations proved to be substantially parallel, consistent with the hypothesis of a prevailing dispersion of favorable dominant alleles in the two parental inbreds. The importance of the level of dominance in affecting the relationship between level of heterozygosity and phenotypic performance was also shown in a computer simulation study conducted by BERNARDO (1992). The results of our study are also consistent with the ones of STUBER *et al.* (1992) who found that the highest correlation coefficients were the ones involving GY. The high relationship detected with GY in that study and in ours is also consistent with the high genetic complexity of the trait. As emphasized by STUBER *et al.* (1992), traits controlled by only one or a few loci are expected to show very low correlation coefficients between phenotypic performance and the level of heterozygosity across the genome. Significant correlation coefficients found in our study for several traits (especially GY) should be also ascribed to the fact that dominance was always unidirectional, as revealed by QTL analysis. YU *et al.* (1997) found in rice a poor relationship between marker heterozygosity and trait expression; they ascribed this finding to the detected bidirectional dominance, *i.e.*, to the cancellation between positive and negative dominance effects of the QTL controlling the trait.

The highest correlation coefficients for most traits were obtained when considering the DIFF data set as a dependent variable, likely because of drawbacks in the other approaches. In fact, when considering the performance of TC(B) and TC(H) hybrids *per se*, the effect of heterozygosity can be, to some extent, masked by the effects of dominant alleles from each tester. As for correlation coefficients obtained with the H_{mp} data sets, heterosis is calculated on the basis of the performance of RILs *per se*, *i.e.*, of the less vigorous and stable materials, thus leading to some inconsistent estimates.

Genomic regions most involved in heterosis: Some regions were identified as showing overlaps among QTL controlling the traits with greater heterosis, namely SW, PH, NK, and GY (see Figure 2, summarizing the information of Table 6). Of the 21 QTL for GY, 12 (57%) overlapped with QTL for NK and 13 (62%) overlapped with QTL for PH. Moreover, when these three traits were considered together, eight overlaps were noted (38%). Five overlaps were also noted among QTL for GY and QTL for SW (24%). Overlaps involving QTL for the four traits were noted in two cases, *i.e.*, at bin 4.10 and bin 8.03. In addition, the prevailing gene action for the QTL involved in all such overlaps was overdominance, indicating that the best genotype was the heterozygote. Even though the role of linkage among different QTL controlling the traits cannot be dismissed, it is reasonable to assume that at least some of these overlaps were due to a pleiotropic action of the underlying genes. In particular, it may be hypothesized that these genes indirectly affect SW, PH, NK, and finally GY through a sequence of causally related events by affecting the overall plant vigor. Also, STUBER *et al.* (1992) found important overlaps among QTL for the traits associated with overall plant vigor, namely ear-leaf area, plant height, and grain yield.

A strong consistency was also found between QTL displaying overdominant effects in our study and in other studies, especially in the work conducted on the material derived from the cross between B73 and Mo17 (Stuber *et al.* 1992; Cockerham and Zeng 1996). Both Mo17 and H99, crossed with B73 in the Stuber *et al.* and in this study, respectively, are classified as members of the LSC heterotic group (Melchinger *et al.* 1991). However, they differ for several traits, including flowering date, plant height, kernel size, and grain yield (Veldboom and Lee 1996).

Some of the QTL detected as overdominant for GY in this work were identified in the Cockerham and Zeng (1996) analysis as well. Whereas five of these QTL fell in the same bin in both studies, in eight other instances the detected overdominant QTL were located in adjacent bins. Nevertheless, these latter QTL could correspond to the same genes, because of different marker genome coverage in the two studies. Three of the regions that we classified as overdominant did not show prominent overdominance in Cockerham and Zeng (1996). However, for two of them, in bins 3.05 and 6.01, high overdominant regions were found in a completely unrelated population (Lu *et al.* 2003), close to marker *phi153* (mapping in bin 3.05) and to *bnlg426* (mapping in bin 6.01). In bin 7.03, we detected overdominant QTL for KW and PH, colocating with overdominant QTL for GY and other traits found by Cockerham and Zeng (1996) (linked to *npi394*) and for GY found by Lu *et al.* (2003) (linked to *bnlg339*).

The consistency between this study and the Cockerham and Zeng (1996) study is noteworthy, since it indicates that most of the same QTL regions control heterosis for growth and yield traits within both our and their materials. These observations could have important implications for future breeding efforts. In fact, the targeted characterization of the chromosomal regions controlling hybrid vigor could allow the selection of particularly favorable heterotic allelic combinations, even in other U. S. genotypes.

The production of advanced mapping populations, such as introgression lines (Semel *et al.* 2006), and near isogenic lines could be devised for the fine mapping of QTL relevant for heterosis. These strategies, complemented by the information from the ongoing Maize Genome Sequencing Project (http://www.maizesequence.org), should allow, in the near future, the identification of candidate genes and candidate regulatory regions crucial for the establishment of heterosis.

Conclusion: In the research presented here, we addressed the genetic basis of heterosis in the material developed from the cross between B73 and H99 maize inbred lines. Both classical quantitative genetic and QTL analyses revealed a high level of heterosis for many of the traits analyzed, especially for those related to plant vigor and yield, *i.e.*, for PH, SW, GY, and its component NK. The utilization of a triple testcross design, together with QTL detection procedures, allowed us not only to identify several QTL contributing to heterosis, but also to estimate the principal mode of action of such QTL. A remarkable aspect of our results is the consistency of the classical and QTL approaches. We demonstrated that in our material heterosis was mainly due to allelic interaction (dominance at various levels), with nonallelic interactions (epistasis) playing a less important role. For the three traits showing the highest level of heterosis (GY, NK, and SW) the average degree of dominance was >1. Furthermore, most of the identified QTL controlling heterosis were in the overdominance range, with unidirectional dominance. The high relationships between the level of heterozygosity at marker loci and phenotypic performance, found for the traits exhibiting the highest heterotic response, emphasized the importance of unidirectional dominance in affecting heterosis. Some chromosomal regions showed overlaps among QTL for GY and QTL for the other heterotic traits, suggesting pleiotropy as a likely causal basis of such overlaps. However, a population's dimension and genetic map density did not allow us to distinguish between true and pseudo-overdominance, and this is still a major limitation in our understanding of heterosis.

Recently, extensive transcription profiling comparing inbred lines and their hybrids by means of DNA microarray technology in maize and mouse (Cui *et al.* 2006; Guo *et al.* 2006; Stupar and Springer 2006; Swanson-Wagner *et al.* 2006) and allelic transcription variation due to *cis*-regulatory elements in maize (Guo *et al.* 2004) indicated that transcriptional regulation and transcriptional overdominance could play an important role as molecular mechanisms establishing hybrid vigor. A finer characterization of QTL with a high degree of dominance might provide crucial information for solving this long-standing controversy regarding overdominance. As a contribution, we are currently producing near-isogenic lines for the QTL that we reported here as having the highest degree of dominance, with the intent of fine mapping and possibly cloning them.

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