

The Genetic Basis of Heterosis: Multiparental Quantitative Trait Loci Mapping Reveals Contrasted Levels of Apparent Overdominance Among Traits of Agronomical Interest in Maize (*Zea mays* L.)

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ABSTRACT Understanding the genetic bases underlying heterosis is a major issue in maize (*Zea mays* L.). We extended the North Carolina design III (NCIII) by using three populations of recombinant inbred lines derived from three parental lines belonging to different heterotic pools, crossed with each parental line to obtain nine families of hybrids. A total of 1253 hybrids were evaluated for grain moisture, silking date, plant height, and grain yield. Quantitative trait loci (QTL) mapping was carried out on the six families obtained from crosses to parental lines following the “classical” NCIII method and with a multiparental connected model on the global design, adding the three families obtained from crosses to the nonparental line. Results of the QTL detection highlighted that most of the QTL detected for grain yield displayed apparent overdominance effects and limited differences between heterozygous genotypes, whereas for grain moisture predominance of additive effects was observed. For plant height and silking date results were intermediate. Except for grain yield, most of the QTL identified showed significant additive-by-additive epistatic interactions. High correlation observed between heterosis and the heterozygosity of hybrids at markers confirms the complex genetic basis and the role of dominance in heterosis. An important proportion of QTL detected were located close to the centromeres. We hypothesized that the lower recombination in these regions favors the detection of (i) linked QTL in repulsion phase, leading to apparent overdominance for heterotic traits and (ii) linked QTL in coupling phase, reinforcing apparent additive effects of linked QTL for the other traits.

THE harmful effect of inbreeding and the higher vigor of hybrids compared to their inbred parents were first observed by Darwin (1876) and then described in maize by East (1908) and Shull (1908). The superiority of the hybrids was later defined as heterosis by Shull (1914). The comprehension and prediction of this phenomenon, widely used in agriculture, are a major research issue. Hybrid breeding programs would benefit substantially from a reliable way to predict hybrid phenotypes through a better understanding

of the underlying genetic bases of heterosis. Three major genetic mechanisms explaining heterosis have been proposed, dominance, overdominance, and epistasis, but their relative importance is not clearly elucidated (see Lamkey and Edwards 1999 for review). The dominance hypothesis invokes the masking of deleterious recessive alleles of one parent by dominant (or partially dominant) alleles of the second parent, to explain the hybrid vigor of the F_1 (Davenport 1908; Bruce 1910; Jones 1917). The overdominance hypothesis postulates that heterosis is due to the superiority *per se* of heterozygous genotype compared to either parental homozygous genotype at individual loci (Hull 1945; Crow 1948). Tight linkage between loci with favorable dominant alleles in repulsion phase may lead to an apparent overdominance of the chromosome region, which is referred to as pseudo-overdominance (Jones 1917; Crow *et al.* 1952). Finally, positive epistatic interactions between nonallelic genes can also

Copyright © 2012 by the Genetics Society of America
doi: 10.1534/genetics.111.133447

Manuscript received July 29, 2011; accepted for publication November 20, 2011
Available freely online through the author-supported open access option.

Supporting information is available online at <http://www.genetics.org/content/suppl/2011/11/30/genetics.111.133447.DC1>.

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contribute to heterosis [epistasis hypothesis (Richey 1942; Powers 1944; Jinks and Jones 1958; Williams 1959)].

During the 20th century, a lot of studies were conducted to investigate the genetic bases of heterosis, particularly in maize, which is one of the most heterotic cultivated plants. Before the advent of molecular markers, two main kinds of experimental approaches were developed for that purpose (see Lamkey and Edwards 1999 for review). Generation means analyses rely on the comparison of genetic effects estimated from the means of different generations. Variance components analyses partition the genetic variance into its components due to additive, dominance, and epistatic effects. Comstock and Robinson (1952) devised one of the most powerful and widely used experimental designs that can be used for partitioning the genetic variance: the North Carolina design III (NCIII). This design is based on the backcross of a random sample of F_2 individuals to the two inbred lines from which they were derived. It provides an estimation of the average level of dominance of genes affecting the evaluated traits. A question raised by the early variance component analyses was the effect of linkage on estimates of additive and dominance variance. In the presence of linkage in repulsion phase, additive variance is expected to be underestimated whereas dominance variance is overestimated. The estimates of average degree of dominance from these early studies were indeed usually in the overdominant range, suggesting overdominance of at least some loci. However, average degrees of dominance estimated from F_2 populations randomly mated for several generations to permit genetic recombination and approach linkage equilibrium were always smaller than the estimates from nonrandom mated F_2 populations and usually in the partial to complete dominance range (Hallauer and Miranda 1981). These results convinced most of the scientists that much of the observed overdominance was probably pseudo-overdominance due to linkage bias (Lamkey and Edwards 1999).

The development of molecular markers for genetic analyses was a major step toward the analysis of the type of gene action underlying heterosis. Two main approaches have been used: (i) the investigation of the relationship between heterosis and genetic divergence between parental lines and (ii) QTL mapping for the identification of chromosomal segments involved in heterosis. Indeed, several studies have reported positive correlation between genetic distance between parents based on molecular markers and hybrid vigor, either in diverse (Lee *et al.* 1989; Smith *et al.* 1990; Liu *et al.* 2002; Barbosa *et al.* 2003) or in linkage mapping (Stuber *et al.* 1992; Frascaroli *et al.* 2007) populations. This relationship has been theorized by Charcosset *et al.* (1991), Charcosset and Essioux (1994), and Bernardo (1992), who showed that high correlations can be observed only if (i) heterosis is due to a substantial number of loci displaying dominance effects and (ii) genetic markers used to compute distance display linkage disequilibrium with loci involved in heterosis. Variation in linkage disequilibrium among genetic groups explains why distance-based prediction approaches

are of limited efficiency for hybrids between lines issued from different groups (Melchinger 1999). Regarding the second approach, Stuber *et al.* (1992) conducted the first experiment in plants aimed at localizing quantitative trait loci (QTL) involved in the variation of heterosis. It corresponds to a modified NCIII where F_3 plants coming from an initial B73 \times Mo17 cross have been backcrossed to each parental line. They analyzed each backcross series of progenies separately. They observed QTL at which the heterozygous genotype was significantly superior to both homozygous genotypes and concluded that overdominance (or pseudo-overdominance) was the major cause of heterosis in grain yield. Later on, Cockerham and Zeng (1996) developed a statistical framework for NCIII analysis using the backcross progenies with both parents for QTL mapping and demonstrated that estimates for additive and dominance effects are mixed with epistatic effects. Reanalyzing data from Stuber *et al.* (1992) with their statistical theory, they concluded that heterosis for grain yield was mainly due to dominance and hypothesized that linkage between QTL in repulsion phase, leading possibly to the cancellation of additive effects and aggregation of dominance effects, can have created the pseudo-overdominance cases observed in their analyses. Moreover, Graham *et al.* (1997), using near-isogenic lines (NILs), dissected an overdominant QTL of chromosome 5, first identified by Stuber *et al.* (1992), into two tightly linked dominant QTL in repulsion phase. Since then, other studies relying on molecular markers and NCIII [based on the backcross of F_2 , F_3 , or recombinant inbred lines (RILs) to their parental lines] have been performed. Schön *et al.* (2010) have summarized the results of previous NCIII studies and reanalyzed three of them, Stuber *et al.* (1992), Lu *et al.* (2003), and Frascaroli *et al.* (2007), using the same QTL detection approach as in Frascaroli *et al.* (2007), *i.e.*, using linear combinations of performances in the two backcross progenies to detect directly additive and dominance effects. Their results point to pseudo-overdominance as a major cause for heterosis in maize; they found no QTL with significant epistatic effect. They revealed a surprising congruency of heterotic QTL positions for grain yield among studies and found that almost all congruent QTL were located close to the centromeres where recombination is limited and favorable alleles have therefore a higher chance of being in repulsion linkage disequilibrium (McMullen *et al.* 2009). Using another design introduced by Hua *et al.* (2003) on rice, the “immortalized F_2 ” (iF_2) population developed from pair crosses of RILs, Tang *et al.* (2007, 2010) concluded that dominance effects at heterotic loci as well as additive-by-additive epistatic interactions played an important role in the genetic basis of heterosis in maize.

In addition to maize, heterosis has been studied in other crops like rice and pronounced differences have been found between the two species. Hua *et al.* (2003), using an iF_2 population demonstrated that heterotic effects at the single-locus level, in combination with dominant-by-dominant epistatic interactions, can adequately explain the genetic basis of heterosis in rice. Garcia *et al.* (2008), using NCIII designs compared maize and rice and concluded that additive-by-

additive epistasis contributed to heterosis in rice, whereas dominance was a major cause of heterosis in maize. Altogether, QTL mapping studies in maize and rice suggest that genetic bases underlying heterosis could be different, depending on the reproductive biology of the species. Maize, which is an allogamous species, might have accumulated more deleterious recessive alleles than rice, since they are masked by their corresponding dominant counterparts. In autogamous species, the maintenance of dominance effects must be less important and epistasis seems to be more frequent (Garcia *et al.* 2008). This subject has, however, not been sufficiently documented to reach a clear conclusion and further investigations are needed to detect QTL contributing to heterosis and analyze their effects.

QTL mapping of heterotic loci has usually been carried out in biparental populations. The aim of the present work is to study the genetic basis of heterosis for several traits, using an extension of the NCIII approach, investigating a larger genetic diversity. This extension of design III is based on three initial inbred parents, each belonging to a different heterotic group, intercrossed following a half-diallel scheme to form recombinant populations that are then crossed to all parents, either related to the population or not. In this article, we first analyzed our design as a set of “classical” NCIII populations, excluding unrelated crosses, following the approach first developed by Cockerham and Zeng (1996) and extended by Melchinger *et al.* (2007) to the analysis of RILs. We then analyzed our complete design with a multiparental connected model explicitly modeling the effect of all genotypes at a given QTL and, finally, compared these two approaches. This extension of the NCIII enables us to study heterosis in families deriving from both related and unrelated parents and to compare not only contrasts between homozygous and heterozygous genotypes but also contrasts between heterozygous genotypes at each locus. It also provides a means to test for epistatic effects between individual QTL and the genetic background.

Materials and Methods

Plant material

Three connected populations of RILs were developed at INRA le Moulon from three inbred lines representative of complementary heterotic groups widely used in the 1980s and early 1990s: a line from the European flint group (F2), a line from the iodent group (Io), and a line from the early dent group (F252). Material development was described in Causse *et al.* (1996). These populations were named D (deriving from the cross of F2 and Io), E (from F2 and F252), and G (from Io and F252) and were composed of 145, 113, and 144 RILs, respectively. All the RILs were crossed to the three initial parental lines to produce nine families of test-cross progenies. Seeds were produced in three isolated plots using each parental line as the male parent. This experimental material can be seen as three connected NCIII designs obtained after cross-

ing each of the three RIL populations to the parental lines they originated from and three supplementary families obtained after crossing the same RIL populations to the unrelated parent (RILs from F2 × F252 crossed with Io, RILs from F2 × Io crossed with F252, and finally RILs from F252 × Io crossed with F2). The three parental lines (F2, F252, and Io) and the three F₁ hybrids produced from these lines were also crossed with the three parental lines and used as checks.

Phenotypic evaluation and statistical analyses

A total of 1253 experimental hybrids were evaluated in three locations in France in 1993 (Mons, Gif-sur-Yvette, and Clermont-Ferrand) and one location (Gif-sur-Yvette) in 1994. In each location, field trials were divided into 18 blocks of 72 plots each. As far as possible, the three hybrids deriving from the same RIL were placed in the same block. Within each block hybrids and parental checks between unrelated parents and between related parents were grouped separately in 3 subblocks of 24 plots each (2 for related crosses and 1 for unrelated crosses) to avoid competition effects caused by expected vigor differences. In addition to the parental hybrids, two commercial hybrids were also used as checks: Aviso in the “related” subblocks and DEA in the “unrelated” subblocks, according to their vigor. Unlike in tested hybrids, checks were replicated in each location, 3–6 times for parental checks and >50 times for commercial hybrids following an incomplete block design. Individual plots consisted of two rows ~5 m long according to the location. Plant density was 9–10 plants/m² following the usual practice of the site. Silking date (in number of days after May 1, which is considered the average date of sowing for the different trials, evaluated as the date at which 50% of the plants exhibited silks) and plant height (evaluated as the average of 5 plants) were recorded in all locations for 1993 experiments. Grain moisture (percent) and grain yield adjusted to 0% grain moisture ($q \cdot \text{ha}^{-1}$) were measured at harvest in all environments (location and year combination). For grain yield, Clermont-Ferrand was excluded from the analysis because in this southern location, the late genotypes obtained after crossing with the late parental line Io were clearly favored and this masked the effect of heterosis.

Statistical analysis

Grain yield was adjusted regarding plot density, for each cross and environment. Plots with <60% of the expected density were excluded from the grain yield and moisture data sets. For each trait, we performed analyses of variances with ASReml-R (Butler *et al.* 2009). We applied the model

$$Y_{ijkl} = \mu + \varphi_j + \varphi(\alpha)_{ji} + \beta_l + \beta(\gamma)_{lk} + \delta_m + \varepsilon_{ijkl},$$

where Y_{ijkl} indicates performance of genotype i within hybrid family j evaluated on the block k of the environment l . The block effect (γ_k), considered as random, was nested in a fixed environment (corresponding to the four location and year combinations) effect (β_l). All the levels of block within the

environment effect were considered as independent. In this model, we considered separately tested genotypes and checks. The genotype effect (α_i) was nested in a fixed family effect (φ_j) and treated as random whereas check effect (δ_m) was considered as fixed. Using a similar procedure to that in Cullis *et al.* (1989) and Moreau *et al.* (1999), all the checks were attributed the same level for the family and genotype effects, whereas, symmetrically, all the genotypes of the nine families were attributed the same level for the check effect. A genetic variance was estimated for each family. ε_{ijkl} was the residual error.

For each trait we computed family, parental lines, and hybrid means and their standard error, as well as broad sense heritabilities for each family on the global experimental design using the formula

$$h_j^2 = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_e^2/L_j N_j} \quad (1)$$

with σ_g^2 being the genetic variance of the genotypes of the family j and σ_e^2 the residual variance (as only one replication was present in each environment, the genotype \times environment interaction and the plot error variances are confounded in σ_e^2). L_j is the average number of environments and N_j the average number of replicates per environment for the family j (N_j was slightly inferior to 1 due to missing data).

Heterosis and epistasis tests on means

Midparent heterosis can be tested on the basis of the contrast between F_1 hybrid mean and average performance of the parental lines. This contrast was tested with a Student's t -test with d d.f.,

$$\frac{H_{12} - 0.5(P1 + P2)}{\sqrt{1/n_h + (1/4)(1/n_{p1} + 1/n_{p2}) \sigma_e^2}}, \quad (2)$$

where d is the number of degrees of freedom involved in the estimation of σ_e^2 , σ_e^2 is the residual variance estimated in the global analysis of phenotypic data, and H_{12} is the adjusted mean of the F_1 hybrid derived from parental lines 1 and 2. $P1$ and $P2$ are, respectively, the adjusted means of parental lines 1 and 2. n_h , n_{p1} , and n_{p2} are, respectively, the replicate numbers of F_1 hybrid and parental lines $P1$ and $P2$.

Likewise, epistasis can be tested on the basis of adjusted means of the parental and hybrid checks. Indeed, if there is no epistasis, the mean of F_1 hybrids obtained after crossing parental lines 1 and 2 with a third one must be equal to that of the corresponding three-way hybrid. This contrast was also tested with a Student's t -test with d d.f.,

$$\frac{H_{12 \times T} - 0.5(H_{1 \times T} + H_{2 \times T})}{\sqrt{1/n_{h \times T} + (1/4)(1/n_{p1 \times T} + 1/n_{p2 \times T}) \sigma_e^2}}, \quad (3)$$

where d and σ_e^2 are the same as in Equation 2. $H_{12 \times T}$ is the adjusted mean of the F_1 hybrid derived from parental lines 1

and 2, crossed with the third parental line used as a tester (T). $H_{1 \times T}$ and $H_{2 \times T}$ are, respectively, the adjusted means of parental lines 1 and 2 crossed with the same tester (T). $n_{h \times T}$, $n_{p1 \times T}$, and $n_{p2 \times T}$ are, respectively, the replicate numbers of three-way hybrids and F_1 hybrids derived from parental lines 1 and 2.

NCIII statistical analyses

For each of the three NCIIIs, two linear transformations, called Z_1 and Z_2 , corresponding to augmented additive and dominance effects, respectively, were carried out on the basis of adjusted data (for block effects) in each environment. H_1 and H_2 are the phenotypic observations of progenies of each RIL with the parental inbreds 1 and 2, Z_1 is the trait mean across each pair of progenies ($Z_1 = (H_1 + H_2)/2$), and Z_2 is the half difference between each pair of progenies ($Z_2 = (H_1 - H_2)/2$) (Schön *et al.* 2010, adapted from Cockerham and Zeng 1996 and Melchinger *et al.* 2007). We then performed analyses of variance on these linear transformations, using a model including a fixed environment effect and a random genotypic effect. Estimates of the genotypic [$\sigma_g^2(Z_1)$ and $\sigma_g^2(Z_2)$] and residual variance [$\sigma_e^2(Z_1)$ and $\sigma_e^2(Z_2)$] and broad sense heritabilities were calculated for each population D, E, and G (using the formula above). On the basis of the genotypic variance, we estimated the augmented degree of dominance ($\bar{D}^* = \sqrt{\sigma_g^2(Z_2)/\sigma_g^2(Z_1)}$). Finally, global means adjusted for environment effect were computed for Z_1 and Z_2 .

Genotyping and linkage maps

We used 212 microsatellite markers on population D, 225 on population E, and 187 on population G, leading to a total of 288 SSRs polymorphic in at least one population (*i.e.*, an average density of >1 marker every 10 cM). Electrophoreses were performed on 4% Metaphor agarose gels. Segregation distortion was tested for each marker and a few markers were discarded on the basis of this information. Linkage maps were built with Mapmaker software version 3.0b (Lander *et al.* 1987), using a LOD threshold of 3.0 to define linkage groups. Markers were ordered using multipoint analysis and orders on each chromosome were then checked by the "ripple" option. Map distances were obtained with the Haldane mapping function (Haldane 1919). First, one genetic map by population was constructed and then a consensus map was established by considering nonsegregating loci in a population as missing data (the consensus map is available in [Supporting Information, Figure S1](#)). Physical positions of the SSR markers and centromeric markers (cent1–cent10) were retrieved from MaizeGDB to project centromere positions on our genetic map.

Markers were also used to compute the modified Rogers distance (Rogers 1972) between each RIL and each of the parental lines from which they derived. This distance is an estimator of the level of heterozygosity of the hybrid obtained by crossing this RIL to one of the parental lines. This information was compared to (i) hybrid phenotypic performance and (ii) Z_2 transformation.

QTL detection on NCIII designs

QTL analyses were conducted on Z_1 and Z_2 linear transformations of each trait, using a modified version of MCQTL software (Jourjon *et al.* 2005). This software performed QTL detection using an iterative composite-interval mapping (iQTLm) approach (Charcosset *et al.* 2000). In a first step, markers associated with the studied trait were selected as cofactors and used to detect QTL by composite-interval mapping (CIM). The QTL positions identified were then used as cofactors in a new CIM mapping to refine QTL positions. The model stopped after convergence of the QTL positions. A multipopulation model was used to jointly analyze the three NCIII designs. In this analysis, all three populations were used for QTL detection but considered as independent (disconnected model) and locus effects were nested within populations,

$$y = Jm + W_q h_q + \sum_{c \neq q} W_c h_c + e, \quad (\text{model 1})$$

where y was a column vector of performances (Z_1 or Z_2) of the N RIL individuals of the global design coming from P populations (D, E, and G, and thus $P = 3$ in this case). J was the $N \times P$ matrix whose elements were 0 or 1 according to whether individual i belonged to the p th population, and m was a $P \times 1$ vector of population-specific effects. W_q or W_c was a $N \times 2P$ matrix containing the expected number of allele k carried by the inbred line i at the QTL q (or cofactor c) position given the marker data. The total number of allele effects estimated in the three NCIII designs was $2P$. By definition, on a given line of W_q or W_c only the two elements corresponding to alleles segregating within the population of individual i can be nonnull and their sum equals 2. h_q or h_c was a $2P \times 1$ column vector of the within-population allelic effects at QTL q (or cofactor c), and the sum of the effects of the two alleles segregating in a given population was constrained to be zero. e was the vector of the residuals.

Genotypic probabilities were computed every 2 cM. We used a significance threshold ($-\log_{10}(P\text{-value})$) determined by 2000 permutation tests to reach a global type I risk of 10% genome-wide. This threshold was fixed at 3.4 for grain moisture, plant height, and grain yield and at 3.6 for silking date. Cofactors were selected by forward regression and the analysis stopped in a 20-cM window around the other cofactors detected on the studied chromosome. The cofactor threshold was calculated as the significance threshold of QTL $- 0.5$. At the end of the detection process, the QTL confidence intervals were estimated on the basis of a 2-LOD unit fall.

QTL detection on the global design

Environment and block effects were estimated and subtracted from the raw plot values to compute an adjusted mean for each genotype for QTL mapping. Multiparental connected models on adjusted means of testcross progenies for grain yield, grain moisture, flowering time, and plant height were performed on the global design (the total of the

nine families, the six included in the three NCIIIs and the three unrelated crosses), using the model

$$y = Jm + X_q^* g_q^* + \sum_{c \neq q} X_c^* g_c^* + e, \quad (\text{model 2})$$

where y was a column vector of performances of the N test-cross progenies of the global design coming from F families ($F = 9$ in our case). J was an $N \times F$ matrix whose elements were 0 or 1 according to whether individual i belonged to family f , and m was an $F \times 1$ vector of family-specific effects. X_q^* or X_c^* was an $N \times K$ matrix ($K = K^* + (K^* \times (K^* - 1)/2)$) (K^* being the number of parental inbreds, three in our case), with K^* columns containing the expected number of allele k given the marker data for each individual i and $K^* \times (K^* - 1)/2$ columns containing the probability of individual i being a heterozygote at the QTL q (or cofactor c). g_q^* or g_c^* was a $K \times 1$ column vector of the K^* allele additive effects at QTL q (or cofactor c) (the sum of the additive effects of the two alleles segregating in a given family was constrained to be zero) and the $K^* \times (K^* - 1)/2 = 3$ dominance effects associated with hybrid genotypes at QTL q (or cofactor c). e was the vector of the residuals. Note that this model is comparable to the one used by Rebai *et al.* (1997) but is extended here to (i) the joint analysis of inbred and noninbred families and (ii) multi-QTL mapping.

Parameters of QTL detections (QTL significance and cofactor thresholds, cofactor window, confidence intervals, etc.) were estimated or chosen as were the parameters of the NCIII detection. The QTL significance threshold was fixed at 3.5 for grain moisture, silking date, and plant height and at 4 for grain yield.

Tests of additive and dominance effects

As MCQTL tests only the global effect of QTL, additive and dominance effects were tested for each significant QTL using the incidence matrices built by MCQTL, with programs developed in R (R Development Core Team 2011). Significance levels of the additive and dominance effects were tested at each QTL with Fisher's tests based on nested models.

Epistasis tests

Similarly we developed programs in R to test the QTL-by-genetic background interactions and pairwise QTL-by-QTL interactions as in Blanc *et al.* (2006).

QTL-by-genetic background interactions: Multipopulation connected analyses (model 2) assume that one allele has the same effect over populations, whereas in multipopulation disconnected analyses the allelic effects are assumed to be different in each family so $2F$ effects need to be estimated (F being as previously the number of families). Comparison between the two models enables one to test for QTL-by-genetic background interaction. These interactions were tested using the model

Table 1 Heterosis test based on parental lines and F₁ hybrids performance

Parental lines and F ₁ hybrids	Grain moisture (%)		Silking date (days)		Plant height (cm)		Grain yield (q · ha ⁻¹)	
	Mean ^a ± SE ^b	Heterosis (%)	Mean ± SE	Heterosis (%)	Mean ± SE	Heterosis (%)	Mean ± SE	Heterosis (%)
lo	33.68 ± 0.35		93.77 ± 0.40		170.84 ± 9.21		25.41 ± 7.30	
F2	22.6 ± 0.35		84.26 ± 0.35		145.96 ± 9.21		11.95 ± 7.30	
F252	16.59 ± 0.42		80.66 ± 0.35		172.69 ± 9.21		30.37 ± 8.21	
F2 × lo	27.28 ± 0.18	-3.06	76.77 ± 0.18	-13.76***	225.14 ± 4.61	42.13***	91.42 ± 3.65	389.40***
F2 × F252	21.35 ± 0.18	8.96**	70.48 ± 0.19	-14.53***	215.74 ± 4.88	35.41***	76.57 ± 3.86	261.86***
F252 × lo	23.26 ± 0.18	-7.46**	78.47 ± 0.18	-10.03***	235.05 ± 4.61	36.84***	83.83 ± 3.65	200.57***

Hybrids from unrelated parents are given in boldface type. ***P* ≤ 0.01, ****P* ≤ 0.001.

^a Overall locations adjusted mean.

^b Standard error.

$$y = Jm + X_q g_q + \sum_{c \neq q} X_c^* g_c^* + e, \quad (\text{model 3})$$

where *y*, *J*, *m*, *X_c^{*}*, *g_c^{*}* and *e* were as described in model 2. *X_q* was an *N* × 2*F* matrix containing the expected genotype of the hybrid *i* at the QTL *q* position given the marker data. The total number of genotype effects in the global design was 2*F*. By definition, on a given line of *X_q* or *X_c* only the two elements corresponding to the possible genotypes of individual *i* in the population could be nonnull and their sum equaled 1. *g_q*, which was a 2*F* × 1 column vector of the within-population effects at QTL *q* and the sum of the effects of the two genotypes segregating in a given population, was constrained to be zero.

The QTL-by-genetic background interaction sum of squares, calculated as the difference between the residual sum of squares in model 2 [RSS₍₂₎] and in model 3 [RSS₍₃₎], has *F* - *K* - 1 d.f. (since in the disconnected model, *F* families allow the estimation of *F* contrasts and in the connected model *K* - 1 independent effects are estimated). Using models 2 and 3 made it possible to perform a Fisher's test for QTL-by-genetic background interaction,

$$F \text{ test} = \frac{(RSS_{(2)} - RSS_{(3)}) / (F - (K - 1))}{RSS_{(3)} / (N - 2F - (K - 1)C)},$$

where *N*, *P*, and *K* were as previously defined and *C* was the number of cofactor QTL treated as connected.

The model used for this test corresponded to the final model 2 reached after convergence, with final estimated positions of QTL. The same positions were used in model 3. Note that this test follows an *F* distribution under the hypothesis that the estimated QTL positions are the true ones.

QTL-by-QTL interactions: Digenic epistasis between all pairs of detected QTL was tested by comparing model 2 to the following one,

$$y = Jm + X_q^* g_q^* + X_{q'}^* g_{q'}^* + X_{qq'}^* g_{qq'}^* + \sum_{c \neq q, q'} X_c^* g_c^* + e, \quad (\text{model 4})$$

where elements indexed with *q* (or *q'*) corresponded to the first (or second) locus involved in the interaction. *X_{qq'}^{*}* was

a *N* × *K*² matrix equal to the horizontal direct product of each column of *X_q^{*}* by each column of *X_{q'}^{*}*, and *g_{qq'}^{*}* was a *K*² × 1 vector of the effects of the interaction between QTL *q* and *q'*. The other parameters were defined as in model 2. The interaction has (*K* - 1)² d.f.

Each QTL-by-QTL interaction was partitioned into two components: additive-by-additive interactions and all interaction terms involving dominance effects (i.e., dominant-by-dominant, dominant-by-additive, and additive-by-dominant interactions). Additive-by-additive interactions were tested by comparing model 2 to a model (further called model 5) similar to model 4 except that the epistatic interaction term was limited to the interaction between additive effects (i.e., *X_{qq'}^{*}* was the horizontal direct product of the columns of *X_q^{*}* and *X_{q'}^{*}* corresponding to additive effects). In model 5, *X_{qq'}^{*}* has *K*² columns and (*K*² - 1)² d.f. Interactions involving dominance effects were tested by comparing the residual sum of squares of models 4 and 5.

Results

Statistical analysis revealed that differences among trials (environments) and genotype × environment interactions were significant for most traits (results not shown). However, the genotypic variances were always superior to the genotype × environment ones. Therefore, only mean values across trials are presented and discussed.

Heterosis and epistasis tests on means

Performances of the parental and hybrid checks (Tables 1 and 2) showed that material involving parent F2 generally performed less well than did material involving other parental lines. The F₁ hybrids and three-way hybrids and families deriving from unrelated parents displayed higher values for grain yield and plant height than lines and crosses involving related parents. Tests for midparent heterosis (Table 1) pointed out that, as expected, heterosis for plant height and grain yield was significant and displayed high relative values, up to 42% and 390%, respectively. We also observed significant heterosis toward earliness up to -14% for silking date. For grain moisture, contrasts between F₁ hybrids and mean of parental inbred lines presented variable signs and were not significant for two of three hybrids.

In most of the cases, the contrast between the mean of the F₁ hybrid crossed to the unrelated parent used as a tester

Table 2 Epistasis test based on parental lines, F₁, and three-way hybrids performance

Three-way hybrids	Grain moisture (%)		Silking date (days)		Plant height (cm)		Grain yield ($q \cdot ha^{-1}$)	
	Mean ^a ± SE ^b	Epistasis	Mean ± SE	Epistasis	Mean ± SE	Epistasis	Mean ± SE	Epistasis
loF2 × F2	26.26 ± 0.35	NS	75.94 ± 0.35	***	189.37 ± 9.21	NS	50.72 ± 7.30	NS
loF2 × F252	22.39 ± 0.35	NS	74.27 ± 0.35	NS	226.81 ± 9.21	NS	86.14 ± 7.30	*
loF2 × lo	28.2 ± 0.35	**	82.61 ± 0.35	***	200.3 ± 9.21	NS	65.37 ± 7.30	*
F252F2 × F2	22.41 ± 0.35	NS	72.55 ± 0.35	***	187.48 ± 9.21	NS	52.33 ± 7.30	*
F252F2 × F252	18.79 ± 0.35	NS	74.21 ± 0.35	NS	196.61 ± 9.21	NS	62.82 ± 7.30	**
F252F2 × lo	24.41 ± 0.35	NS	75.43 ± 0.35	***	219.9 ± 9.21	**	89.61 ± 7.30	NS
loF252 × F2	23.72 ± 0.35	NS	71.59 ± 0.35	**	219.62 ± 9.21	NS	83.43 ± 7.30	NS
loF252 × F252	19.9 ± 0.38	NS	77.48 ± 0.35	**	200.31 ± 9.21	NS	68.72 ± 8.21	***
loF252 × lo	26.21 ± 0.38	**	82.81 ± 0.35	***	199.11 ± 9.21	NS	65.61 ± 8.21	***

Hybrids from unrelated parents are given in boldface type. * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$; NS, not significant.

^a Overall locations adjusted mean.

^b Standard error.

and the mean of the corresponding three-way hybrid was significantly different from zero for silking date and grain yield, providing evidence of epistasis for these traits (Table 2). For silking date, epistasis was always negative (data not shown), *i.e.*, leading to a greater precocity of the three-way hybrid than expected from parental hybrid values. For grain yield, epistasis was positive when significant and could not be related to consanguinity. For grain moisture and plant height only a few contrasts showed significant epistasis. For the other contrasts, the lack of difference suggested either the lack of epistatic interactions or the presence of interactions of opposite signs canceling each other out.

Performances of the nine families

A very large variation was observed for all the studied traits among the tested progenies (Table 3). As expected, for plant height and grain yield, populations crossed to their nonparental line performed on average better than the populations crossed to either of their parental lines. For grain moisture and silking date, the trend was not so clear because heterosis is mixed with tester precocity. For all the families, heritabilities were medium to high (>0.58) for all the studied traits except for grain moisture in family D × F2 ($h^2 = 0.30$) and for grain yield in families D × F252 ($h^2 =$

0.15), E × Io ($h^2 = 0.38$), and G × F2 ($h^2 = 0.27$). The latter three families, deriving from unrelated parents, displayed high average performance and low genetic variance relative to that observed for families derived from related parents.

Summary statistics for Z₁ and Z₂ linear transformations

For all populations and all the traits except grain yield, heritability estimates for Z₁ (augmented additive effect) were high ($h^2 > 0.7$) and superior to heritabilities estimated for Z₂ (augmented dominance effect) (Table 4). Estimates of augmented degree of dominance \bar{D}^* were high (and superior to 1) for grain yield (1.31–1.95), medium for plant height (0.51–0.59) and silking date (0.67–0.81), and low for grain moisture (0.29–0.38).

Relation between Z₂ and hybrid heterozygosity

As the correlation between hybrid performance and hybrid heterozygosity depends on both dominance and additive effects, we focused here on the correlation between Z₂ (the augmented dominance effects) and the modified Rogers distance to parent 1. Correlations between hybrid performance and hybrid heterozygosity for all the studied traits are available in Figure S2, Figure S3, Figure S4, and Figure S5. For

Table 3 Performance and broad sense heritabilities of families (population crossed to one of the three parental lines)

Families	Grain moisture (%)		Silking date (days)		Plant height (cm)		Grain yield ($q \cdot ha^{-1}$)	
	Mean ^a ± SE ^b	h^{2c}	Mean ± SE	h^2	Mean ± SE	h^2	Mean ± SE	h^2
D × F2	26.67 ± 0.01	0.30	77.84 ± 0.06	0.83	176.87 ± 1.43	0.82	47.41 ± 0.72	0.72
D × F252	22.26 ± 0.02	0.62	74.55 ± 0.04	0.77	223.25 ± 0.90	0.75	84.47 ± 0.22	0.15
D × lo	28.81 ± 0.03	0.65	82.74 ± 0.05	0.83	197.74 ± 0.98	0.79	71.11 ± 0.55	0.67
E × F2	23.28 ± 0.03	0.71	74.05 ± 0.06	0.85	174.97 ± 1.47	0.83	50.11 ± 0.97	0.80
E × F252	19.1 ± 0.02	0.61	74.57 ± 0.05	0.79	188.9 ± 1.08	0.77	59.68 ± 0.49	0.58
E × lo	25.39 ± 0.03	0.71	76.81 ± 0.04	0.78	218.12 ± 0.93	0.74	86.92 ± 0.32	0.38
G × F2	24.82 ± 0.02	0.64	73.15 ± 0.03	0.78	215.61 ± 0.57	0.66	80.69 ± 0.21	0.27
G × F252	19.72 ± 0.02	0.64	78.21 ± 0.02	0.66	196.56 ± 0.62	0.70	66.57 ± 0.44	0.66
G × lo	26.32 ± 0.03	0.73	83.82 ± 0.03	0.75	196.51 ± 0.73	0.75	64.94 ± 0.57	0.74

Hybrids from unrelated parents are given in boldface type. The nine families correspond to the three RIL populations crossed to the three parental inbred lines (F2, F252, and lo). Population D derives from the cross of lines F2 and lo, population E from F2 and F252, and population G from lo and F252.

^a Overall locations adjusted mean.

^b Standard error.

^c Broad sense heritability.

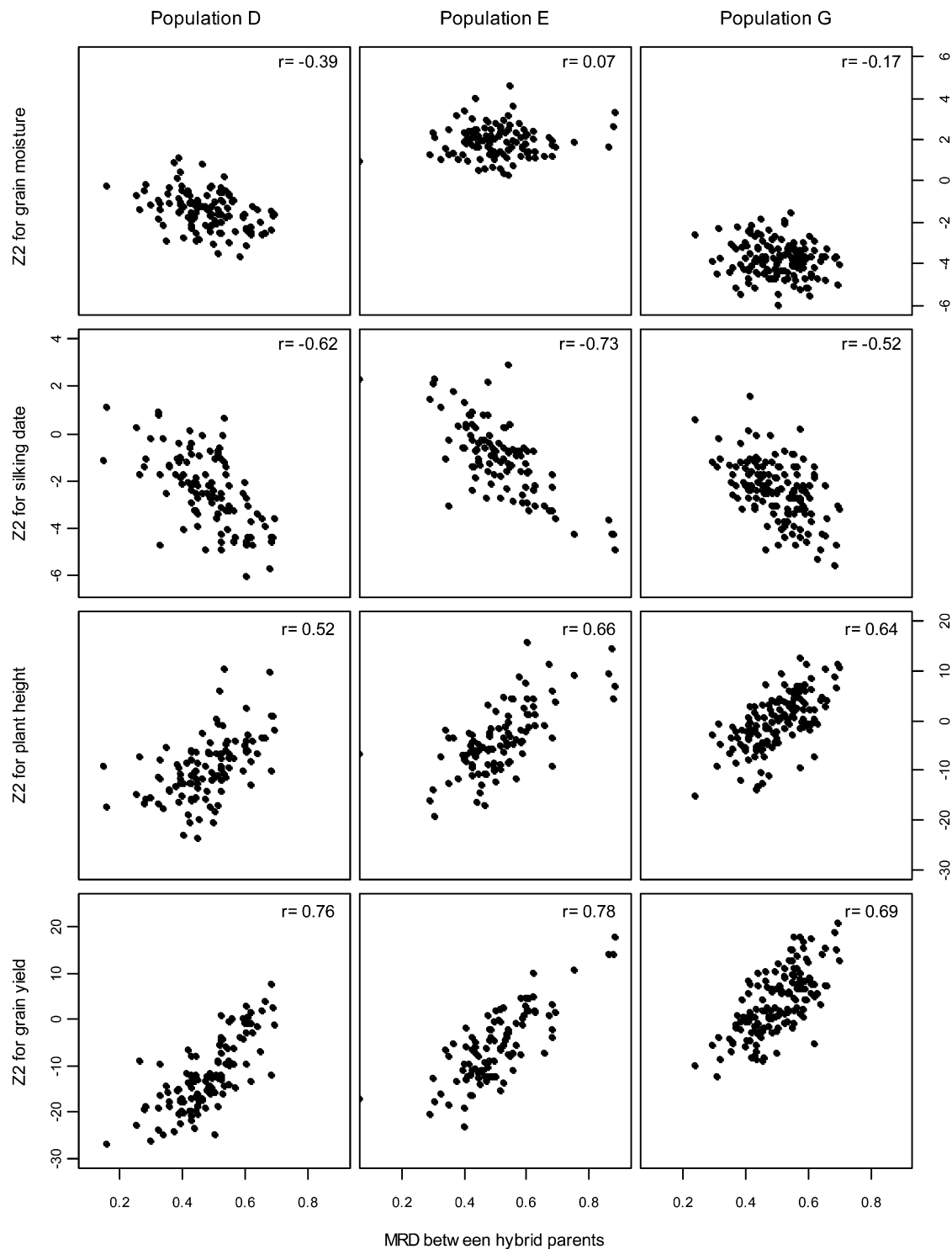


Figure 1 Correlation between augmented dominance effect (Z_2) and modified Rogers distance (MRD^2) to parent 1 for all the studied traits [grain moisture (%), silking date (days), plant height (cm), and grain yield ($g \cdot ha^{-1}$)]. Population D stands for the population deriving from the cross of the parental lines F2 and Io. Population E stands for the population deriving from F2 and F252 and population G stands for the population deriving from Io \times F252.

grain yield, we observed strong positive correlations (ranging from 0.69 to 0.78) between Z_2 and the modified Rogers distance to parent 1 in all the populations studied (Figure 1). Z_2 , for plant height and silking date, displayed the same

kind of relationship with high correlations (ranging, respectively, from 0.52 to 0.66 and from -0.52 to -0.73). Finally, weaker correlation tendencies were observed for grain moisture (ranging from 0.07 to -0.39).

Table 4 Summary statistics by population for linear transformations Z_1 and Z_2

Population		Grain moisture (%)			Silking date (days)		
		D	E	G	D	E	G
Z_1	Mean ^a ± SE ^b	28.44 ± 0.16	18.42 ± 0.15	23.08 ± 0.16	79.43 ± 0.20	74.35 ± 0.19	80.86 ± 0.12
	σ_g^{2c}	2.64	1.86	3.39	3.72	3.65	1.91
	σ_e^{2d}	1.19	2.39	1.4	1.72	1.5	0.98
	h^{2e}	0.87	0.7	0.9	0.85	0.86	0.85
Z_2	Mean ^a ± SE ^b	-1.45 ± 0.08	1.84 ± 0.07	-3.81 ± 0.07	-2.36 ± 0.14	-1.07 ± 0.14	-2.37 ± 0.10
	σ_g^{2c}	0.39	0.16	0.36	1.68	1.79	1.25
	σ_e^{2d}	1	1.45	1.04	1.62	1.06	0.89
	h^{2e}	0.53	0.25	0.56	0.74	0.81	0.8
	\bar{D}^{*f}	0.38	0.29	0.33	0.67	0.7	0.81
Population		Plant height (cm)			Grain yield ($q \cdot ha^{-1}$)		
		D	E	G	D	E	G
Z_1	Mean ^a ± SE ^b	188.82 ± 0.93	181.68 ± 0.96	194.29 ± 0.69	57.58 ± 0.46	57.60 ± 0.56	68.75 ± 0.42
	σ_g^{2c}	83.57	90.19	59.85	12.5	25.13	15.99
	σ_e^{2d}	31.94	39.19	32.22	30.89	27.34	27.75
	h^{2e}	0.88	0.86	0.84	0.48	0.68	0.61
Z_2	Mean ^a ± SE ^b	-9.69 ± 0.61	-4.02 ± 0.60	-0.38 ± 0.44	-12.55 ± 0.69	-5.64 ± 0.67	3.31 ± 0.56
	σ_g^{2c}	21.55	30.77	20.86	47.63	43.06	39.28
	σ_e^{2d}	35.76	28.33	25.23	25.38	23.69	23.69
	h^{2e}	0.62	0.74	0.71	0.81	0.81	0.82
	\bar{D}^{*f}	0.51	0.58	0.59	1.95	1.31	1.57

Population D is derived from the cross of lines F2 and Io, population E from F2 and F252, and population G from Io and F252.

^a Overall locations adjusted mean.

^b Standard error.

^c Genetic variance.

^d Residual variance.

^e Broad sense heritability.

^f Augmented degree of dominance.

QTL detection for NCIII designs

A summary of the QTL detections is presented in Table 5 and Figure 2. Detailed results of QTL detected for NCIII designs are reported in Table S1. The genome scan for grain moisture revealed seven significant QTL for Z_1 that explained 4.8–9.3% of the phenotypic variance and only three QTL for Z_2 with individual R^2 ranging from 5.2 to 7.1%, with one QTL detected for Z_1 congruent with one for Z_2 . A simultaneous fit of all the QTL explained 36% of the phenotypic variance for Z_1 and 17% for Z_2 .

For silking date, the QTL detection on Z_1 identified only two regions with an average phenotypic variance explained of ~6.5% whereas six regions were identified with Z_2 that explained 6.9–15.9% of the phenotypic variance, with no overlapping QTL between Z_1 and Z_2 . The estimated phenotypic variance explained by all the QTL was 12% for Z_1 and 46% for Z_2 .

For plant height, four QTL were detected for Z_1 with individual R^2 ranging from 5.7 to 10.5%. The genome scan for Z_2 revealed seven QTL, which explained from 4.8 to 12.8% of the phenotypic variance. The confidence intervals of four

QTL detected for Z_2 overlapped with the confidence intervals of three QTL detected for Z_1 . The estimated phenotypic variance explained by all the QTL detected was 26% for Z_1 and 44% for Z_2 .

For grain yield, only two QTL were detected for Z_1 with individual R^2 from 4.9 to 6.3% and nine for Z_2 that explained from 5.4 to 12.9% of the phenotypic variance. All the QTL found for Z_1 explained 11% of the phenotypic variance whereas those found for Z_2 explained 53%.

QTL detection on the global design

QTL detected on the global design for all studied traits are reported in Table 6, Table S2, and Figure 2. Between 10 and 15 QTL were detected for each trait, explaining between 34 and 40% of the phenotypic variation. Individual QTL effects were globally low (the QTL with the highest effect explained 7.2% of the phenotypic variance for grain moisture). Different effects were observed according to the trait considered. All of the QTL found for grain moisture presented significant additive effects but only two of them showed significant dominance effects. For silking date and plant height, most

Table 5 QTL detection summary on global and NCIII designs

Trait	QTL detection on NCIII design				QTL detection on the global design			
	QTL detected for Z_1	R^2 (%)	QTL detected for Z_2	R^2 (%)	No. QTL detected	R^2 (%)	QTL with significant additive effects	QTL with significant dominance effects
Grain moisture	7	36	3	17	13	40	13	2
Silking date	2	12	6	46	12	36	11	7
Plant height	4	26	7	44	15	44	15	8
Grain yield	2	11	9	53	10	34	6	10

of the QTL detected displayed additive effects and about half of them also had dominance effects. Oppositely, all the QTL detected for grain yield displayed significant dominance effects and about half of them also had significant additive effects.

No significant QTL-by-QTL global interactions and interactions involving dominance effects were found. Otherwise, almost all the QTL detected for grain moisture, silking date, and plant height exhibited significant digenic additive-by-additive interactions with another QTL (model 5). Still, for grain yield only two QTL seem to interact. Only three QTL detected (one for silking date and two for plant height) presented significant interactions with the genetic background (model 3).

Comparison between QTL detections on NCIII and on the global design

About 70% of the QTL detected on the global design overlapped with QTL detected for linear transformations Z_1 and Z_2 (Table 6 and Figure 2). Of the 50 regions highlighted by the QTL detection on the global design, 13 were not revealed by Z_1 and Z_2 detections. Likewise, of the 35 regions detected for Z_1 and Z_2 , 6 were not revealed by the detection on the global design. For grain moisture, a majority of QTL were detected for Z_1 , which is consistent with the fact that all the QTL detected for this trait on the global design showed significant additive effects and only 2 of 13 showed significant dominance effects. For grain yield, most of the QTL in NCIII analysis were detected for Z_2 and all the QTL revealed by the

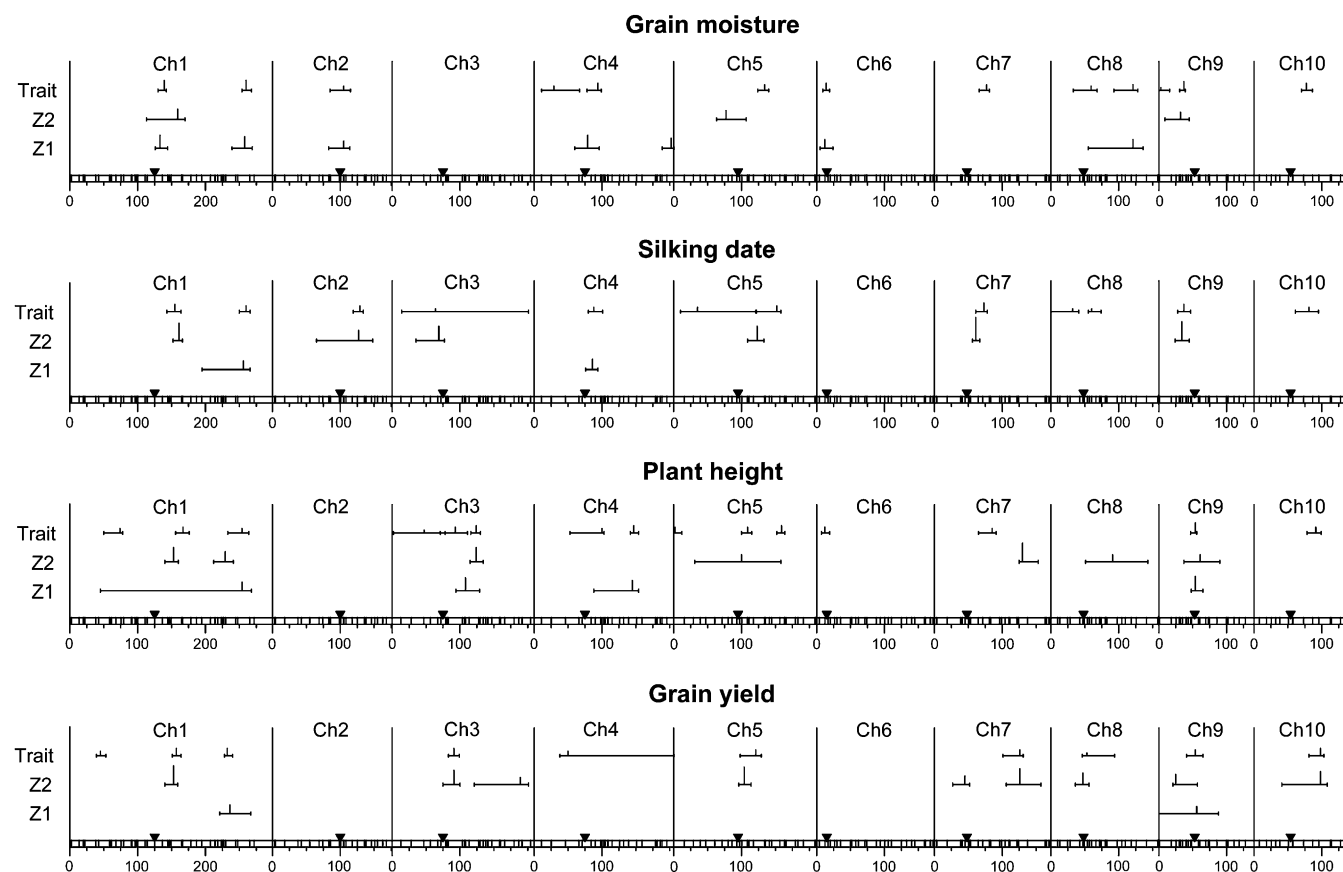


Figure 2 QTL projection for the global design (Trait) and the three NCIII designs (Z_1 and Z_2) for grain moisture, silking date, plant height, and grain yield. Each QTL is displayed by one horizontal line bound by two vertical lines representing the confidence interval and a vertical line proportional to the QTL R^2 symbolizing the QTL position. The solid triangle points to the approximate centromere position.

Table 6 Quantitative trait loci detected on the global design, significance of additive and dominance effects and epistatic interaction

Trait	QTL no.	Chr. no.	Position (cM)	$-\log(P)$ QTL	R^2 (%)	C.I.	Significance of additive effects	Significance of dominance effects	First-order additive \times additive interaction with QTL	Genetic background interaction	Colocalizations with Z_1 and Z_2 QTL
Grain moisture	1	1	139	15.44	7.17	130–142	***	NS	5*		Z_1 and Z_2
	2	1	260	13.95	6.57	254–268	***	NS	8*		Z_1
	3	2	105	5.34	2.92	85–115	***	NS	8*		Z_1
	4	4	29	5.62	3.05	11–67	***	NS	7*, 8**, 9**		Z_1
	5	4	94	9.53	4.73	78–99	***	NS	1*, 12*, 13*		Z_1
	6	5	134	6.99	3.65	124–140	***	NS	7**		
	7	6	14	11.14	5.41	9–19	***	NS	4*, 6**		Z_1
	8	7	77	7.15	3.72	66–81	***	NS	2*, 3*, 4**, 9*, 11**		
	9	8	59	6.31	3.35	33–68	***	NS	4**, 8*, 11***, 12***		Z_1
	10	8	121	6.92	3.61	93–128	***	*	11***, 12*		Z_2
	11	9	3	4.30	2.45	0–16	***	NS	8**, 9***, 10***, 12*		Z_2
	12	9	37	12.20	5.85	31–39	***	*	5*, 9***, 10*, 11*		Z_2
	13	10	77	10.37	5.09	70–86	***	NS	5*		
Silking date	1	1	155	8.70	4.58	143–164	***	***	5*, 12*		Z_2
	2	1	260	7.56	4.07	250–266	***	NS	—		Z_1
	3	2	129	7.95	4.24	119–134	***	***	8*, 10*, 12*		Z_2
	4	3	64	2.80	1.84	14–201	NS	***	5*, 7**	**	Z_2
	5	4	88	4.51	2.66	80–101	***	*	1*, 4*, 6**, 7*, 8*, 10*		Z_1
	6	5	35	5.61	3.18	10–121	***	*	5**, 11**		Z_2
	7	5	152	6.82	3.73	122–158	***	NS	4**, 5*, 8**, 9*, 12***		Z_2
	8	7	73	11.55	5.83	61–78	***	***	3*, 5*, 7**		Z_2
	9	8	32	2.81	1.84	0–41	***	NS	7*		
	10	8	60	3.92	2.38	55–74	***	NS	3*, 5*		
	11	9	37	9.59	4.97	28–47	***	***	6**		Z_2
	12	10	81	6.24	3.47	61–95	***	NS	1*, 3*, 7**		
Plant height	1	1	74	5.17	3.02	50–78	***	*	5**, 6*, 10*, 12**		
	2	1	167	6.82	3.79	156–176	***	***	6**, 14*		Z_1 and Z_2
	3	1	254	6.10	3.45	233–264	***	*	5**, 6*		Z_1 and Z_2
	4	3	47	2.43	1.67	2–78	**	NS	9*		
	5	3	93	7.05	3.89	71–111	***	NS	1**, 3**, 9*, 12**, 13**, 14**, 15*		Z_1
	6	3	124	8.42	4.52	116–130	***	NS	2**, 3*, 9*, 13***, 14*, 15*		Z_1 and Z_2
	7	4	100	4.27	2.59	53–103	***	NS	8**, 9*, 11*		Z_1
	8	4	147	9.43	4.97	142–154	***	NS	7**, 11*		Z_1
	9	5	2	8.14	4.39	0–12	***	NS	4*, 5*, 6**, 7*, 11***		
	10	5	109	6.63	3.70	100–115	***	***	1*, 12**	*	Z_2
	11	5	159	9.27	4.90	152–164	***	*	7*, 8***		Z_2
	12	6	12	6.26	3.53	7–19	***	NS	1**, 5**, 6***, 10**, 14**	*	
	13	7	85	6.07	3.44	65–91	***	*	5**, 6**		
	14	9	54	13.86	6.91	47–56	***	***	2*, 5**, 6**		Z_1
	15	10	91	6.78	3.77	78–99	***	*	5*, 6*		
Grain yield	1	1	45	4.48	2.51	39–53	**	***	—		
	2	1	157	9.48	4.67	151–164	NS	***	—		Z_2
	3	1	232	9.43	4.65	228–240	***	***	—		Z_1
	4	3	91	10.35	5.03	83–99	***	***	7*		Z_2
	5	4	50	6.02	3.19	38–206	***	**	—		
	6	5	121	7.58	3.87	98–129	*	***	—		Z_2
	7	7	126	8.59	4.30	101–131	NS	***	4*		Z_2
	8	8	53	3.60	2.11	46–94	NS	***	—		Z_2
	9	9	54	8.23	4.15	41–65	**	***	—		Z_1 and Z_2
	10	10	98	9.35	4.62	81–103	NS	***	—		Z_2

* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$. NS, not significant.

analysis on the global design presented significant dominance effects. More surprisingly, for silking date and plant height, results for the QTL obtained on the NCIII designs and on the global design were not consistent, since a majority of QTL were detected for Z_2 whereas most QTL detected on the global design presented a significant additive effect but only 15 of 27 showed a significant dominance effect. Figure 2 shows that most of the time, QTL found for the NCIII and global designs colocalized. Confidence intervals of almost half of the QTL detected (42 of

90) encompassed the approximate position of the centromere.

QTL effects

Genetic effects of nine representative QTL are presented in Figure 3. For the other QTL detected, the representation of genetic effects is available in Figure S6, Figure S7, Figure S8, and Figure S9). Estimated effects of grain moisture QTL show a clear predominance of additive effects, the heterozygous genotypes being close to the average effect of the

corresponding homozygous genotypes (Figure 3). On the contrary, for yield QTL that all exhibited significant dominance effects, heterozygous effects were always superior to the average of corresponding homozygous effects. They also appeared superior to the best homozygous genotype in most cases (9 of 10), suggesting overdominance effects. No correlation between homozygous and heterozygous values was observed. For plant height and silking date the pattern was rather additive but dominance (and even overdominance) could be observed at some QTL.

Discussion

Heterosis magnitude

Different magnitudes of heterosis were observed for the traits considered in this study. As expected, slight heterosis was observed for grain moisture. Silking date displayed significant although moderate heterosis up to -14% , *i.e.*, toward earliness. Plant height displayed significant midparent heterosis with values up to 42% . The highest heterosis was found for grain yield, for which F_1 hybrids between parental lines exhibited up to 390% midparent heterosis and the hybrid families derived from the cross with the non-parental line always outperformed the hybrid families deriving from related crosses. Heterosis for grain yield in maize reported in the literature ranges from 150 to 300% (Hallauer and Miranda 1981) so that our material displays among the highest values, possibly due to the use of early flowering genetic pools with limited *per se* vigor. This high value might also be explained in part by the plant density, which was the same for the hybrids and the parental lines. As inbred lines are more sensitive to competition than hybrids, heterosis would probably have been lower at a lower density.

Dominance and overdominance

We used linear transformations (Z_1 and Z_2) to get access to augmented additive and dominance effects. The estimate of the augmented degree of dominance \bar{D}^* varied among the traits for all populations studied. It was maximum for grain yield as the \bar{D}^* estimate was always >1 and thus in the overdominance range. These results are in good agreement with those obtained by Schön *et al.* (2010). However, \bar{D}^* does not reflect the type of gene action at the level of individual loci because it is biased by epistasis and linkage between QTL (Melchinger *et al.* 2007). We calculated the correlation between Z_2 for the studied traits and the modified Rogers distance to parent 1. Assuming unidirectional dominance effects, this correlation increases with the linkage relationship between QTL and markers and decreases with the variance of augmented dominance effects among QTL (Melchinger *et al.* 2010). Z_2 transformation for grain yield and plant height was highly correlated with modified Rogers distance to parent 1. These results are consistent with those of Schön *et al.* (2010) and confirmed the relationship observed between hybrid performance for these traits and hybrid heterozygosity (Figure S4 and Figure S5). More surprisingly, we found for silking

date a high correlation between Z_2 and hybrid heterozygosity whereas the relation between silking date and hybrid heterozygosity was confused by the additive effect of the late Io tester (Figure S3). As expected since grain moisture displayed only slight heterosis, no correlation between Z_2 and hybrid heterozygosity was observed for this trait. These results suggest a polygenic genetic architecture with predominant unidirectional dominance effects for grain yield, plant height, and silking date.

The complex genetic basis of the studied traits hypothesized above is corroborated by the important number of QTL detected and their relatively low individual contributions to trait variation. This number appears comparable to the number detected in other studies based on NCIII or testcross designs (Stuber *et al.* 1992; Rebai *et al.* 1997; Melchinger *et al.* 1998; Ajmone Marsan *et al.* 2001; Moreau *et al.* 2004; Frascaroli *et al.* 2007, 2009; Schön *et al.* 2010). QTL detection with the NCIII designs and with our global design was globally consistent. For grain moisture, a majority of QTL were detected for Z_1 . All 13 QTL detected on the global design presented significant additive effects but only two significant dominance effects, confirming the expected additive genetic architecture of this trait. For grain yield, a majority of QTL were detected for Z_2 and all the QTL detected on the global design showed significant dominance and even apparent overdominance effects (Figure 3 and Figure S9). For plant height and silking date, results underline both additive and dominant QTL, in similar proportions. Dominance effects were usually positive for grain yield and plant height but always negative (leading to earlier flowering) for silking date. Still, in the few cases they were significant, their sign was variable for grain moisture. These results are consistent with the positive correlations between Z_2 and the distance to parent 1 we observed for grain yield and plant height as well as the negative one observed for flowering time (Figure 1). They are also consistent with results from Frascaroli *et al.* (2007). Among the studied traits, the proportion of “dominant” QTL was globally coherent with the heterosis level and the augmented degree of dominance of the trait, indicating a good consistency of phenotypic and QTL analyses. The relatively high levels of heterosis and dominance we observed for silking date were not expected. For example, in the study of Frascaroli *et al.* (2007) pollen shedding exhibited only -5% of heterosis, an average degree of dominance of 0.38 , and was one of the traits exhibiting the lowest proportion of QTL with overdominance or dominance effects. This is probably related to the earlier flowering of our material leading to different genetic architectures. Refined phenotyping would be interesting to evaluate to which extent this corresponds to variation in developmental rate *vs.* differences in plant architecture.

Epistasis

Even if dominance (and perhaps overdominance) seems to have a predominant role in heterosis, epistasis may also be involved by complementation between nonhomologous genes

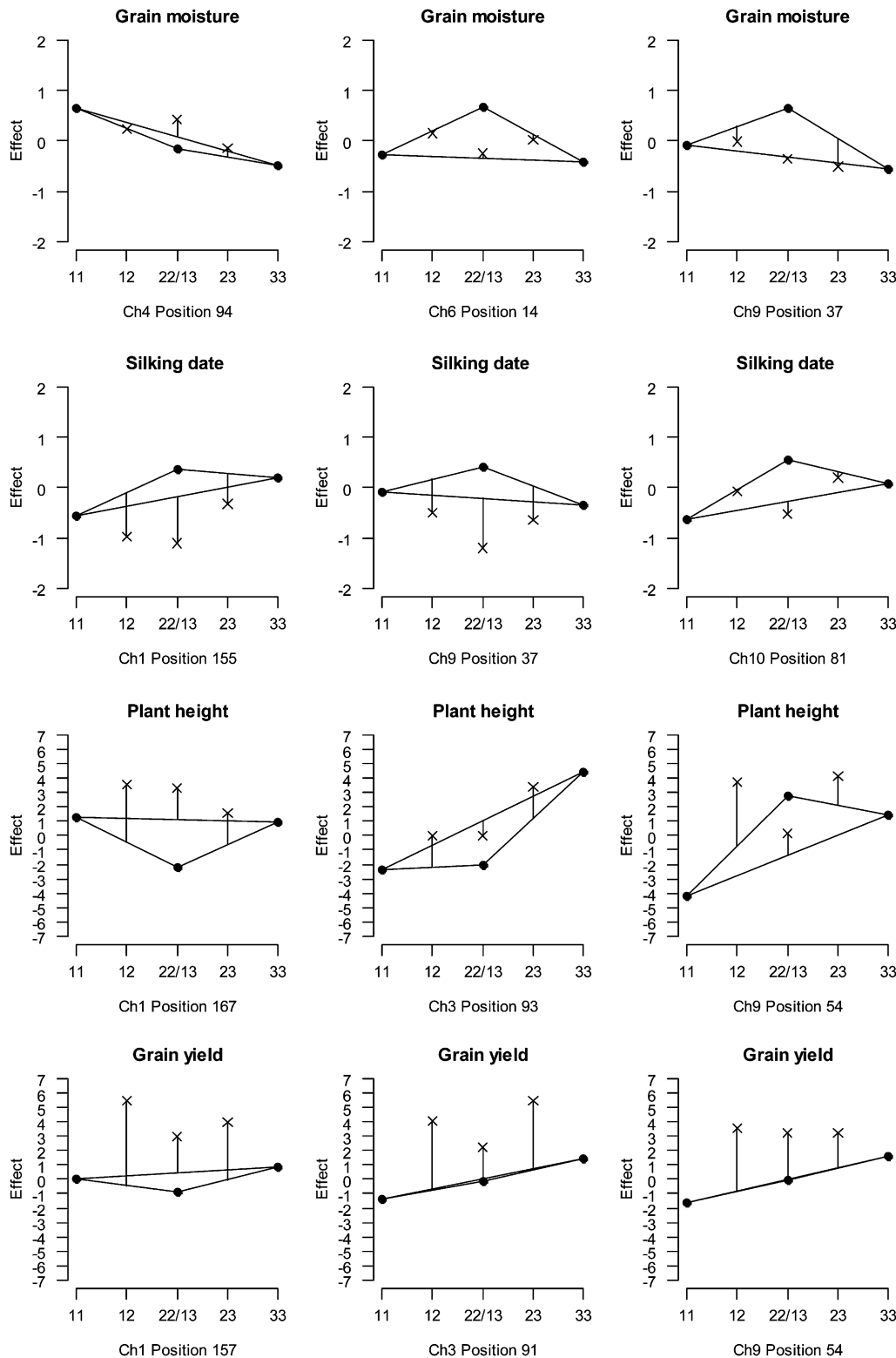


Figure 3 Genetic effects for some representative QTL detected on the global design for grain moisture (%), silking date (days), plant height (cm), and grain yield ($q \cdot ha^{-1}$). Homozygous genotypes are represented by solid circles, and heterozygous genotypes are represented by crosses. Triangle sides join homozygous genotypes whereas vertical lines represent the deviation of each heterozygous genotype from the average of corresponding homozygous genotypes (*i.e.*, dominance effect). 1 indicates the F2 allele, 2 the lo allele, and 3 the F252 allele.

carried by each parent (Gallais 2009, Fiévet *et al.* 2010). Epistatic interactions revealed by molecular markers were reported by some studies in maize, but no clear conclusion was reached concerning the role of epistasis in heterosis. For example, Cockerham and Zeng (1996) found significant epistasis between linked QTL contributing to heterosis for grain

yield. Accordingly, Blanc *et al.* (2006) demonstrated that epistatic interactions between QTL and genetic background were stronger for grain yield than for less complex traits. However, in other studies on maize no significant epistasis was observed (Stuber *et al.* 1992; Lu *et al.* 2003; Frascaroli *et al.* 2007). In our study, significant digenic additive-by-additive interactions

were found for almost all the QTL detected for grain moisture, silking date, and plant height. For grain yield, significant additive-by-additive epistasis was shown for only two QTL and no significant QTL-by-QTL, additive-by-dominant, dominant-by-additive, or dominant-by-dominant interactions were found. In addition, only two QTL detected for plant height and one for silking date seemed to interact with the genetic background. Epistasis tests on parental lines and hybrids means did not reveal the same tendencies since silking date and grain yield were the traits exhibiting the most epistasis. According to these results, we probably do not have enough power to test epistatic interactions at the QTL level when they involve dominance effects. Yan *et al.* (2006) working on grain yield and grain components in $F_{2:3}$ populations of maize showed that most significant epistatic interactions occurred between loci unlinked with the QTL they found. They also established that around half of the QTL detected were involved in epistatic interactions but very seldom interacted with other QTL displaying significant individual effects. They concluded that epistatic interactions between two loci played an equal, if not more important, role than single-locus interaction effects as the genetic basis of heterosis in maize. Although no tool is available to run such analyses in a complex design such as ours, genome-wide scans of epistatic effects certainly deserve further development.

Comparison between classical NCIII and global multiparental analyses

Even if results were globally coherent for analyses carried out on the three NCIII designs and on our global design, several differences were observed. In general, more QTL with smaller confidence intervals (C.I.'s) were detected on the global design. For some QTL with large C.I.'s detected on the three NCIIIs, a QTL with a smaller C.I. or even two QTL were found in our complete design (*e.g.*, grain yield QTL found on chromosome 10, plant height QTL on chromosome 5, and grain moisture QTL on chromosome 8). This is likely to be due to the larger amount of data included in the analysis and the use of connection between populations (Rebai and Goffinet 2000; Jannink *et al.* 2001). However, the analysis of the global design might also be biased by the possible effect of the nonindependence of hybrids. One of the hypotheses for QTL detection with MCQTL is indeed the independence of individuals. In our design the same lines were crossed with the three parental lines and consequently were not independent. However, the cofactors included in the global model of the QTL detection model must partly circumvent this problem and the covariances between the hybrids derived from the same line are expected to be low due to the strong dominance effect.

In contrast to the general trend, five QTL detected for Z_2 transformation did not correspond to any of the QTL detected on the global design (one QTL for grain moisture and two QTL for both plant height and grain yield). This difference can probably be explained by the detection process. By construction, Z_2 depends only on augmented dom-

inance effects and is therefore adjusted for all the augmented additive effects whereas in the analysis of the global design, additive effects are controlled only for the QTL that have been detected. This may diminish power of the global design for detecting QTL exhibiting mainly dominance effects, due to a lower control of the background effect of other QTL.

For traits known to exhibit moderate heterosis such as silking date and plant height, a majority of QTL were found for Z_2 linear transformation and not for Z_1 , whereas on the global design we found that the corresponding QTL showed a significant additive effect and only some of them displayed a significant dominance effect. Melchinger *et al.* (2007) have demonstrated that Z_2 transformation makes it possible to detect QTL of "augmented dominance," meaning that the QTL detected for Z_2 transformation reflect both dominance and additive-by-additive epistatic interactions. We indeed noted that when a QTL detected for Z_2 overlapped a QTL detected on the global design with no significant dominance effect, this QTL almost always presented significant additive-by-additive interactions (*e.g.*, QTL no. 11 for grain moisture, QTL no. 7 for silking date, and QTL no. 6 for plant height). This suggests that, as expected, Z_2 effects are more strongly affected by epistasis than the test of dominance in the global analysis.

Finally, whereas in NCIII, only contrasts between homozygous and heterozygous genotypes are investigated, inclusion of three additional families (obtained after crossing with the nonparental line) in our global design enables us to analyze not only contrasts between homozygous and heterozygous genotypes but also those between different heterozygous ones. For grain yield, we established that differences between homozygous and heterozygous genotypes were always larger than differences between heterozygous genotypes. The former observation was supported by the very low number of QTL detected on the unrelated families when they were analyzed separately (results not shown). This is in agreement with Frascaroli *et al.* (2009) who compared QTL detection on related and unrelated test-cross progenies and concluded that for traits characterized by prevailing dominance-overdominance gene action (such as grain yield) the poorly performing inbred parental line was the most effective tester for QTL detection, whereas the unrelated tester was the least effective. In the case of dominance effects, crosses with a related parent are expected to exhibit a larger genetic variation since their inbreeding coefficient is greater than for progenies from unrelated crosses. The genetic variances were indeed superior for hybrid families derived from crosses with one of their parents for grain yield and plant height (Table 3).

Colocalizations with QTL previously reported for heterosis

For grain yield, 7 of the 10 regions we detected had already been reported for heterosis in the literature. This is notably higher than the level we expected, considering the complexity

of these traits. The region we highlighted on bin 3.05 that presented the highest individual R^2 for grain yield and seemed to be overdominant (Figure 3) was detected as a grain yield QTL with apparent overdominance effect in Lu *et al.* (2003) and in studies by Frascaroli *et al.* (2007). This region was also identified as a heterotic locus (HL) for ear row number in Tang *et al.* (2010). All these studies involved genetic materials unrelated to ours. Pea *et al.* (2009), developing NILs from parental materials used by Frascaroli *et al.* (2007, 2009), confirmed a positive complete dominance effect of this region on yield. They also suggested that this QTL could exert a pleiotropic gene action, first affecting plant height, then ears per plant, and finally grain yield. This hypothesis is consistent with our results, since we found a QTL for plant height in this region.

The region detected on chromosome 1, overlapping with a QTL found for Z_2 , was also reported in studies of Frascaroli *et al.* (2007, 2009) as a QTL for grain yield and number of kernels per plant and in Schön *et al.* (2010) for grain yield. Tang *et al.* (2010) found a HL for 100-kernel weight at the same location. As in Frascaroli *et al.* (2007), this QTL seems to be overdominant in our study (Figure 3).

The region of chromosome 9 detected for grain yield appeared to be involved in plant height, silking date, and grain moisture. This apparent pleiotropic effect is not consistent with results found in the literature. Indeed, Frascaroli *et al.* (2009) found that this region was involved in grain yield variation alone. Likewise, Tang *et al.* (2007, 2010) found two juxtaposed regions involved in ear length and plant height but without overlap. It is noteworthy that our QTL was located in the centromeric region that is characterized by a high ratio between physical and genetic distance, *i.e.*, limited recombination. This QTL region, covering ~ 20 cM, could thus enclose several genes involved in the genetic variation of several traits.

As in Schön *et al.* (2010), many QTL were located close to the centromere. McMullen *et al.* (2009) observed that RILs generally presented higher levels of residual heterozygosity in the centromeric regions. They interpreted this as the consequence of a strong advantage of heterozygosity in these regions, slowing down their fixation during the selfing process. They concluded that this apparent overdominance is most likely the consequence of repulsion between dominant QTL, due to limited recombination in these regions. It can be noted that we found at least as many additive QTL for grain moisture in centromeric regions as dominant QTL for grain yield. This may suggest that we have more power to detect effects in the centromeric region since repulsion leading to apparent overdominance and coupling reinforcing apparent additive effects of linked QTL are favored by the lower recombination (Huang *et al.* 2010). This is supported by Charlesworth and Willis (2009) who suggested that, due to the limited resolution of most QTL studies, linked genes with small individual effects might often appear as a single major QTL, particularly in chromosomal regions with high gene density relative to recombination. As reported by Gallais (2009), natural and even artificial selection create polygenic blocks in

repulsion, and can lead to apparent pleiotropy and overdominance. However, to know whether the overdominance effects observed are true or pseudo-overdominance, it would be worthwhile to explore some of these regions by complementary fine-mapping approaches based on either association genetics adapted to heterosis or near-isogenic materials.

Acknowledgments

We thank Mathilde Causse for contributions to the first steps of this study. We are grateful to colleagues of the Institut National de la Recherche Agronomique experimental units (Le Moulon, Mons, and Clermont-Ferrand) for conducting the phenotypic evaluation. We thank Donald White for the helpful English revision. We are grateful to the two anonymous reviewers and editors for helpful comments.

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Communicating editor: D. W. Threadgill

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Supporting Information

<http://www.genetics.org/content/suppl/2011/11/30/genetics.111.133447.DC1>

The Genetic Basis of Heterosis: Multiparental Quantitative Trait Loci Mapping Reveals Contrasted Levels of Apparent Overdominance Among Traits of Agronomical Interest in Maize (*Zea mays* L.)

A. Larièpe, B. Mangin, S. Jasson, V. Combes, F. Dumas, P. Jamin, C. Lariagon, D. Jolivot, D. Madur, J. Fiévet, A. Gallais, P. Dubreuil, A. Charcosset, and L. Moreau

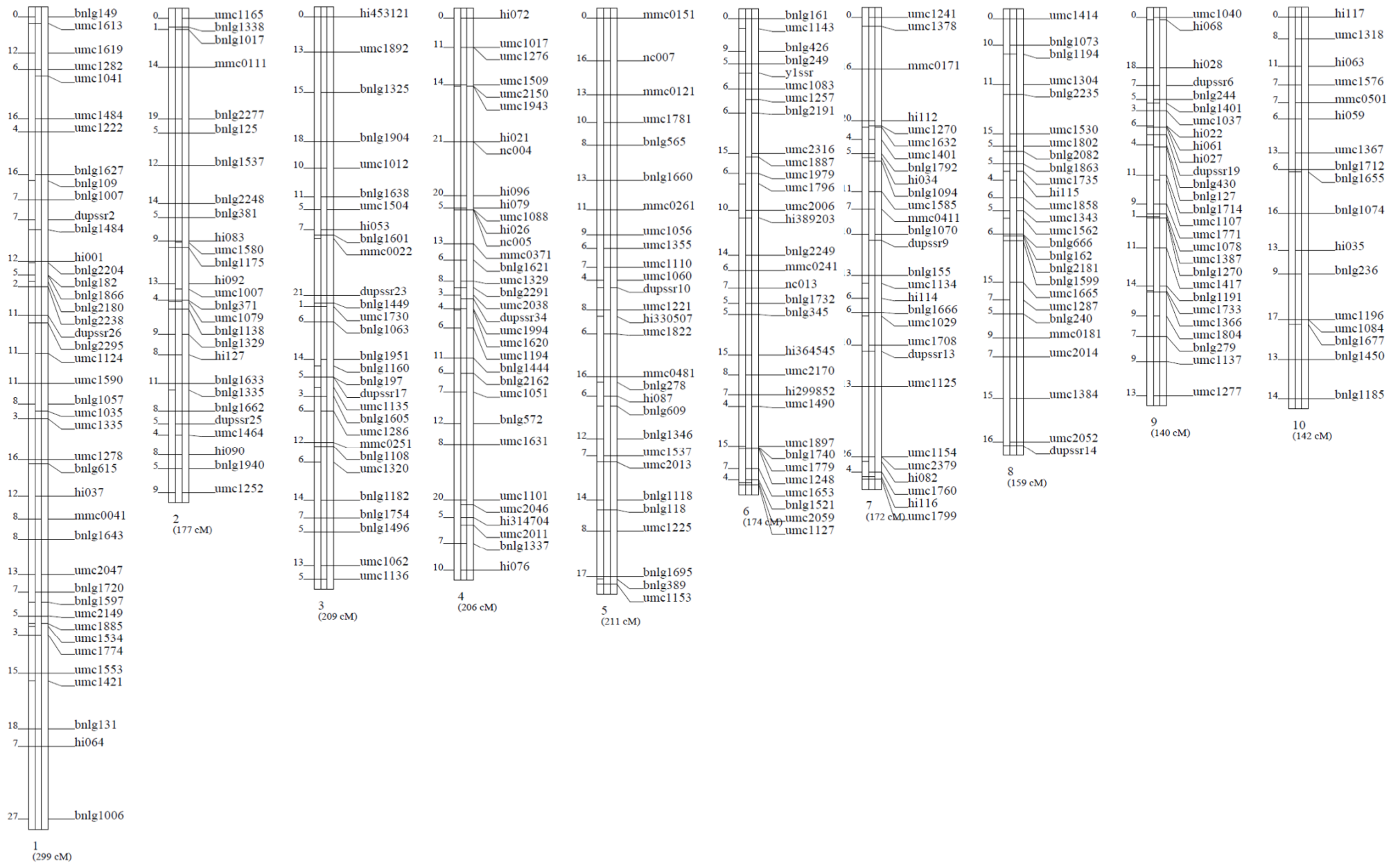


Figure S1 Consensus map of populations D, E and G.

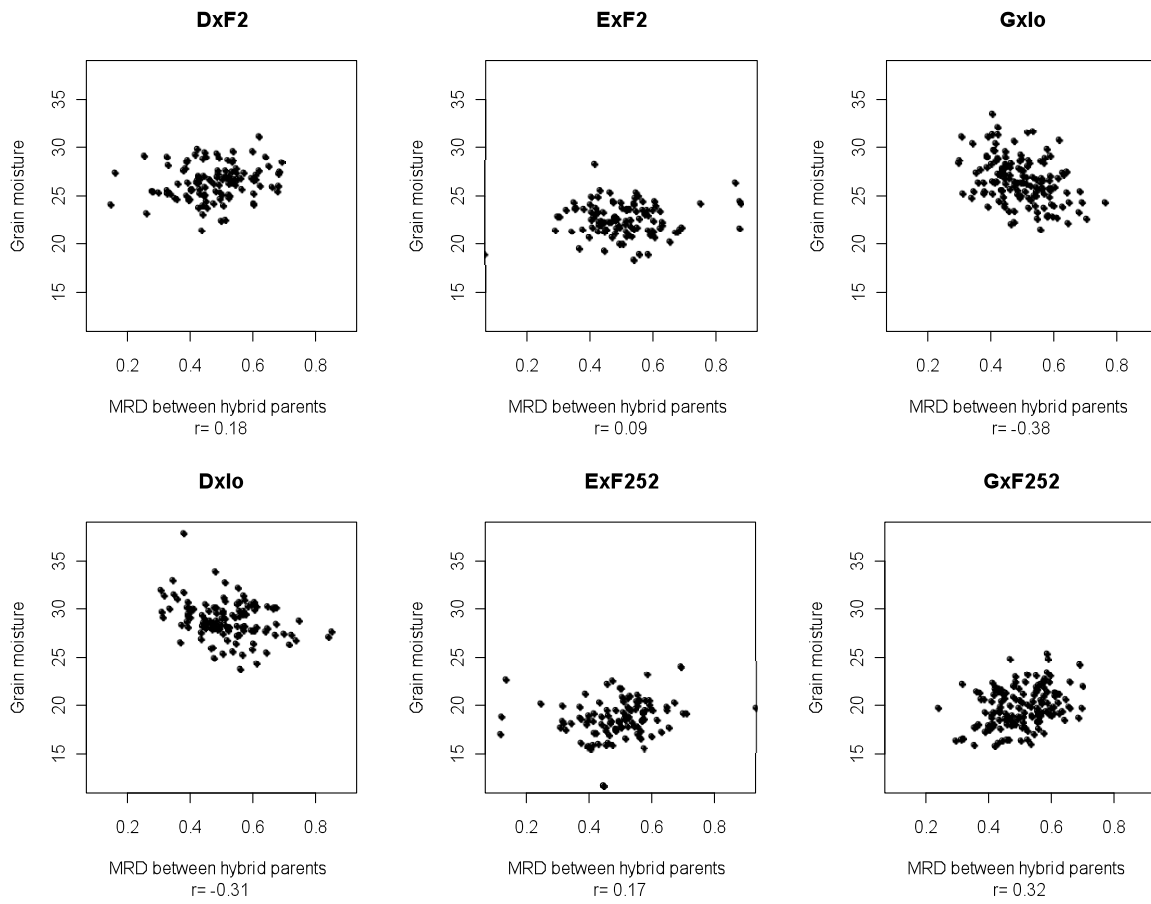


Figure S2 Correlation between hybrid grain moisture (%) and their heterozygosity (estimated using modified Roger's distance (MRD) between hybrid parents).

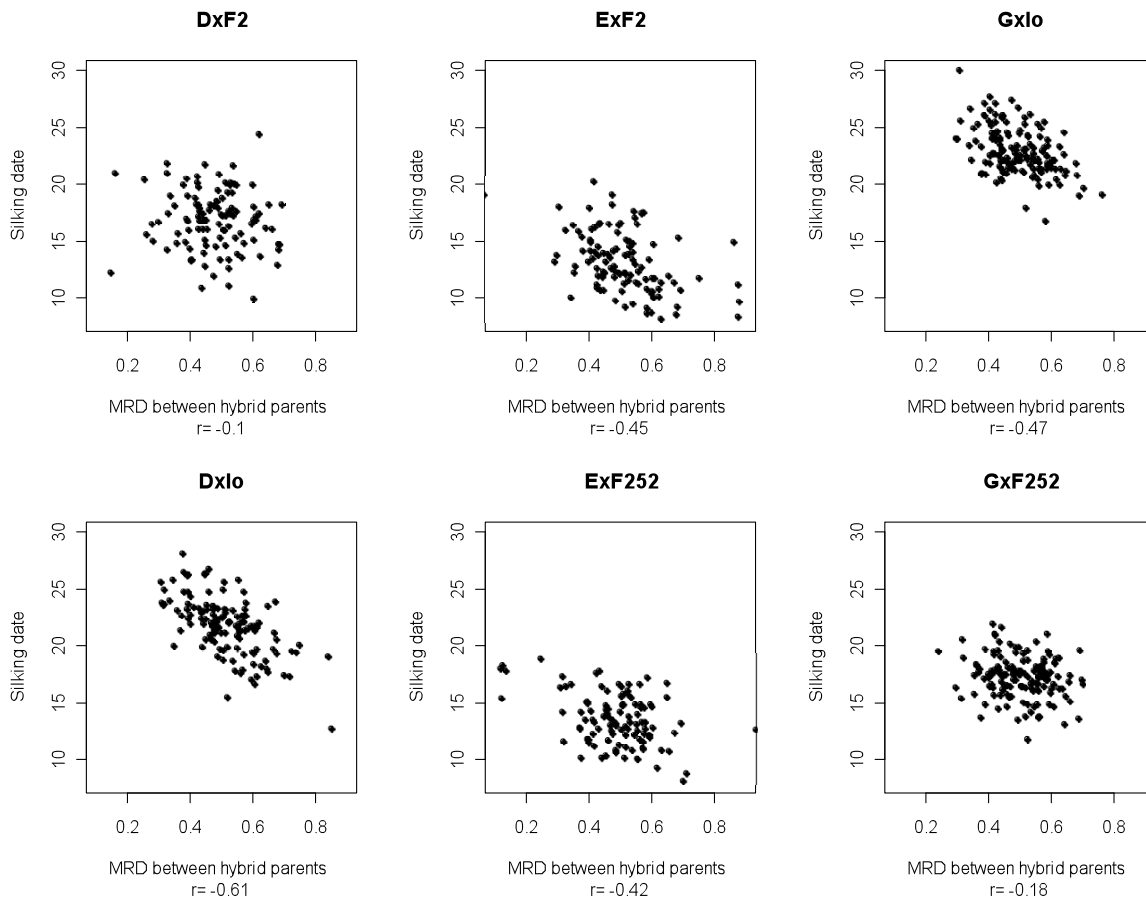


Figure S3 Correlation between hybrid silking date (days) and their heterozygosity (estimated using modified Roger's distance (MRD) between hybrid parents).

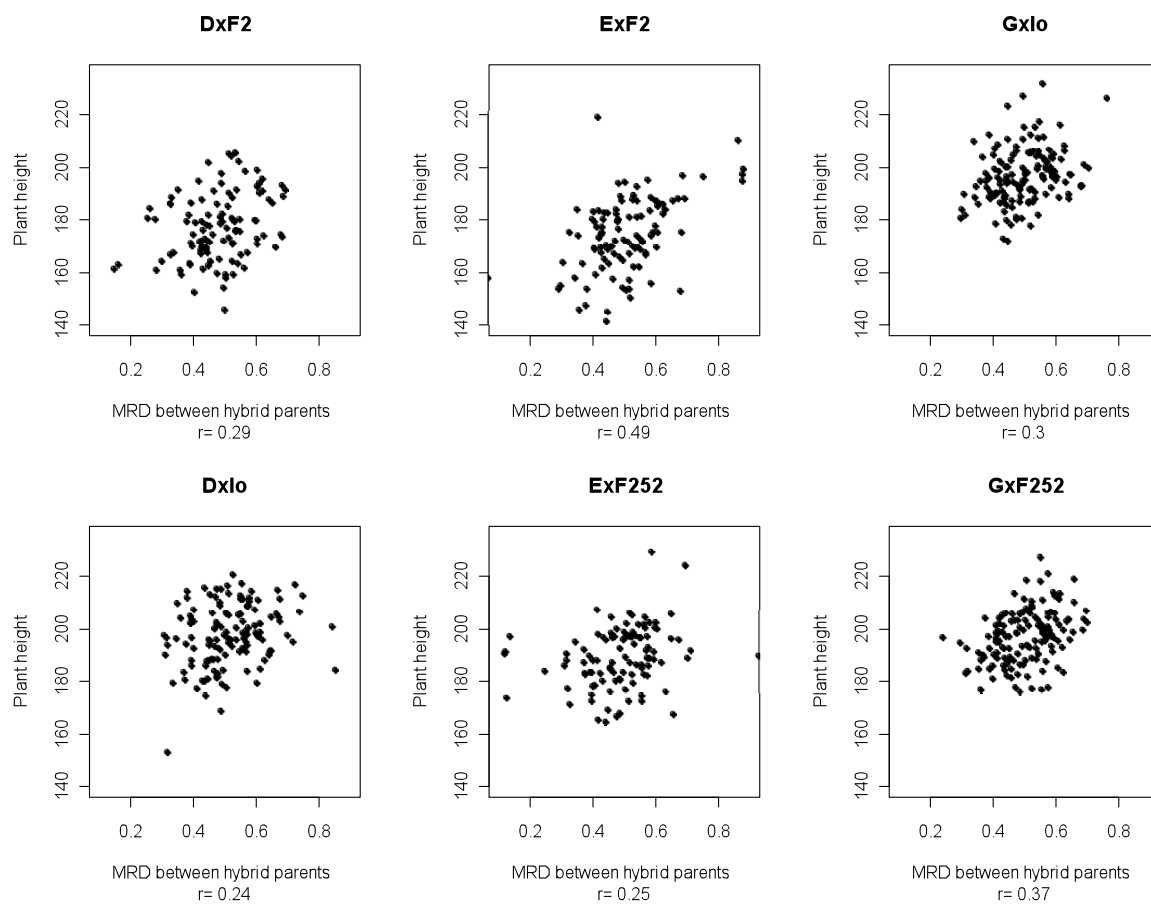


Figure S4 Correlation between hybrid plant height (cm) and their heterozygosity (estimated using modified Roger's distance (MRD) between hybrid parents).

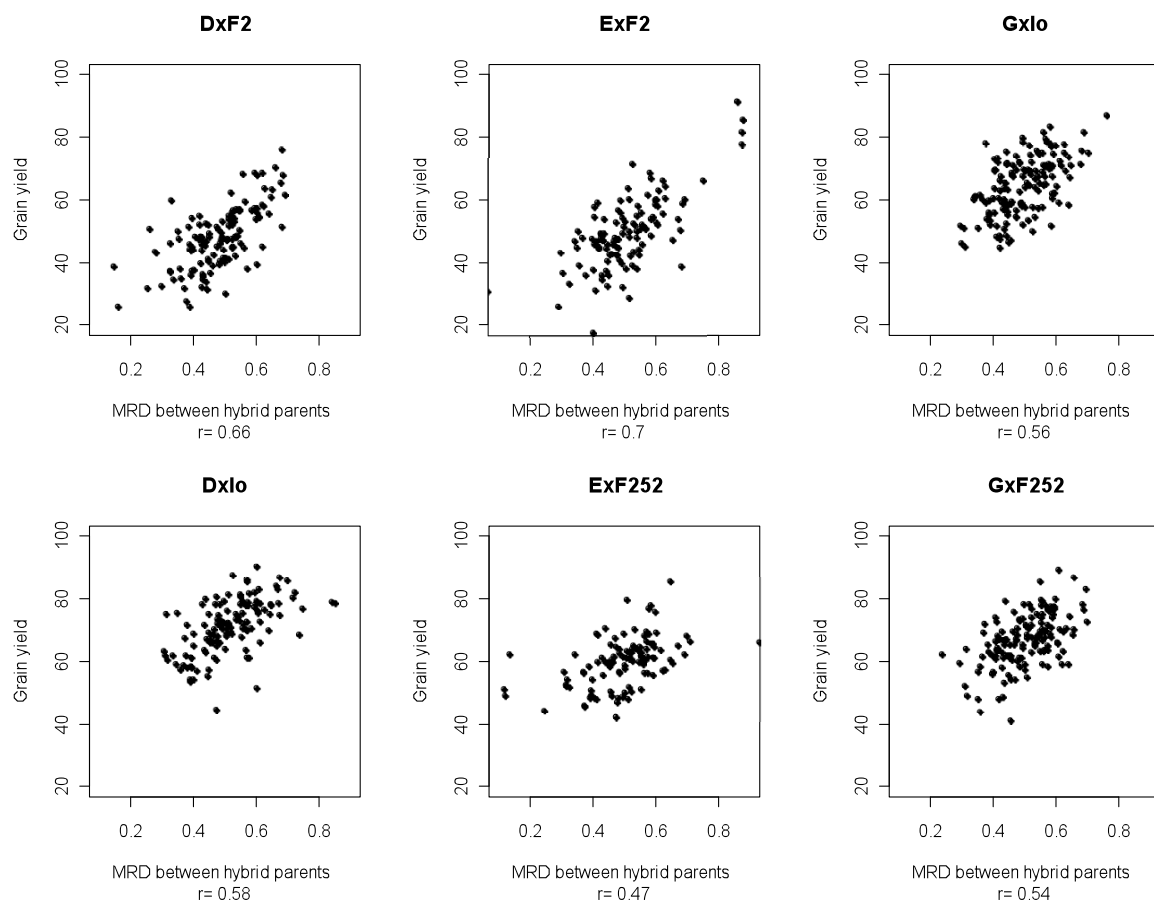


Figure S5 Correlation between hybrid grain yield (q.ha⁻¹) and their heterozygosity (estimated using modified Roger's distance (MRD) between hybrid parents)

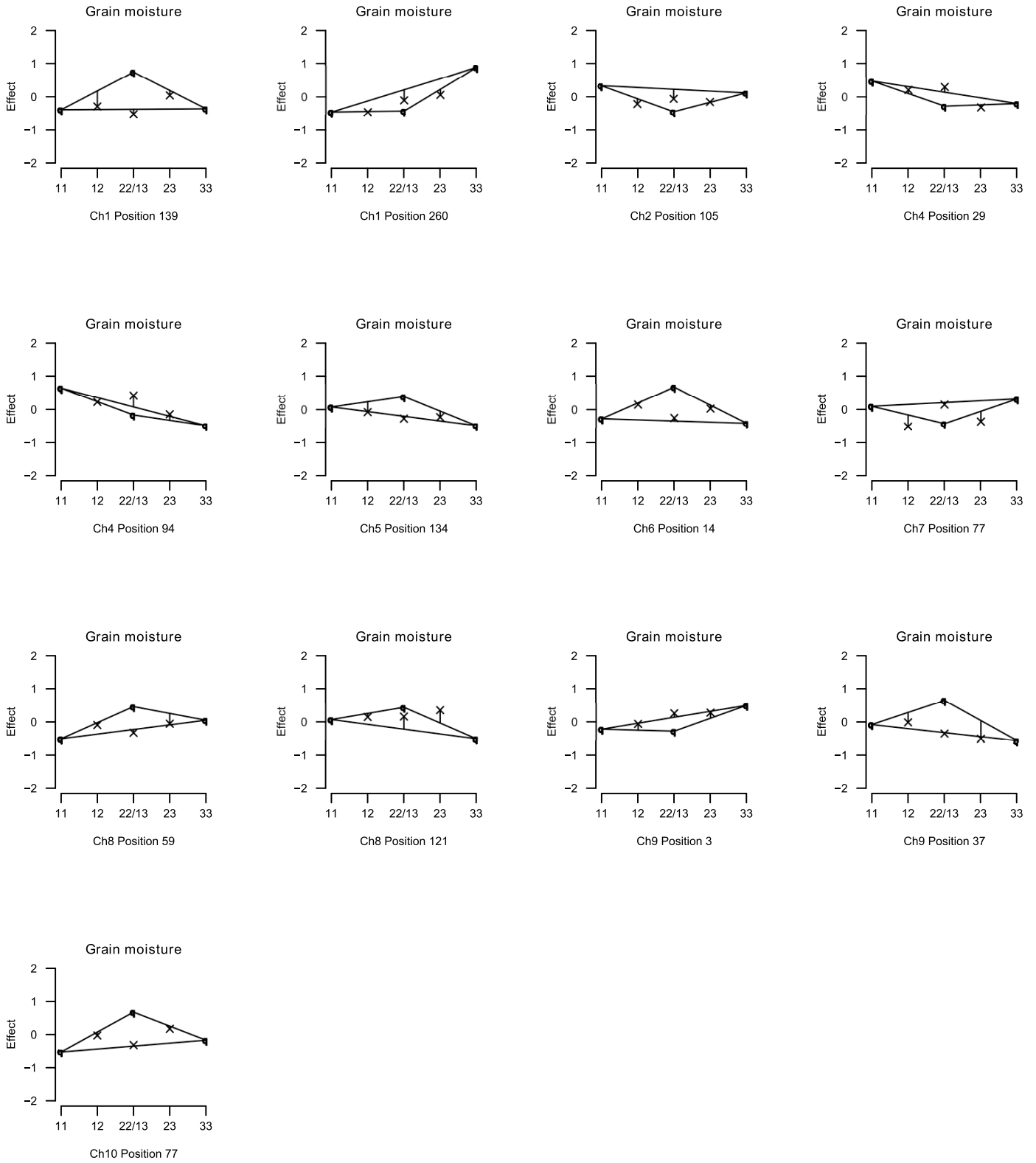


Figure S6 Representation of genetic effects for the QTL detected on the global design for grain moisture (%)

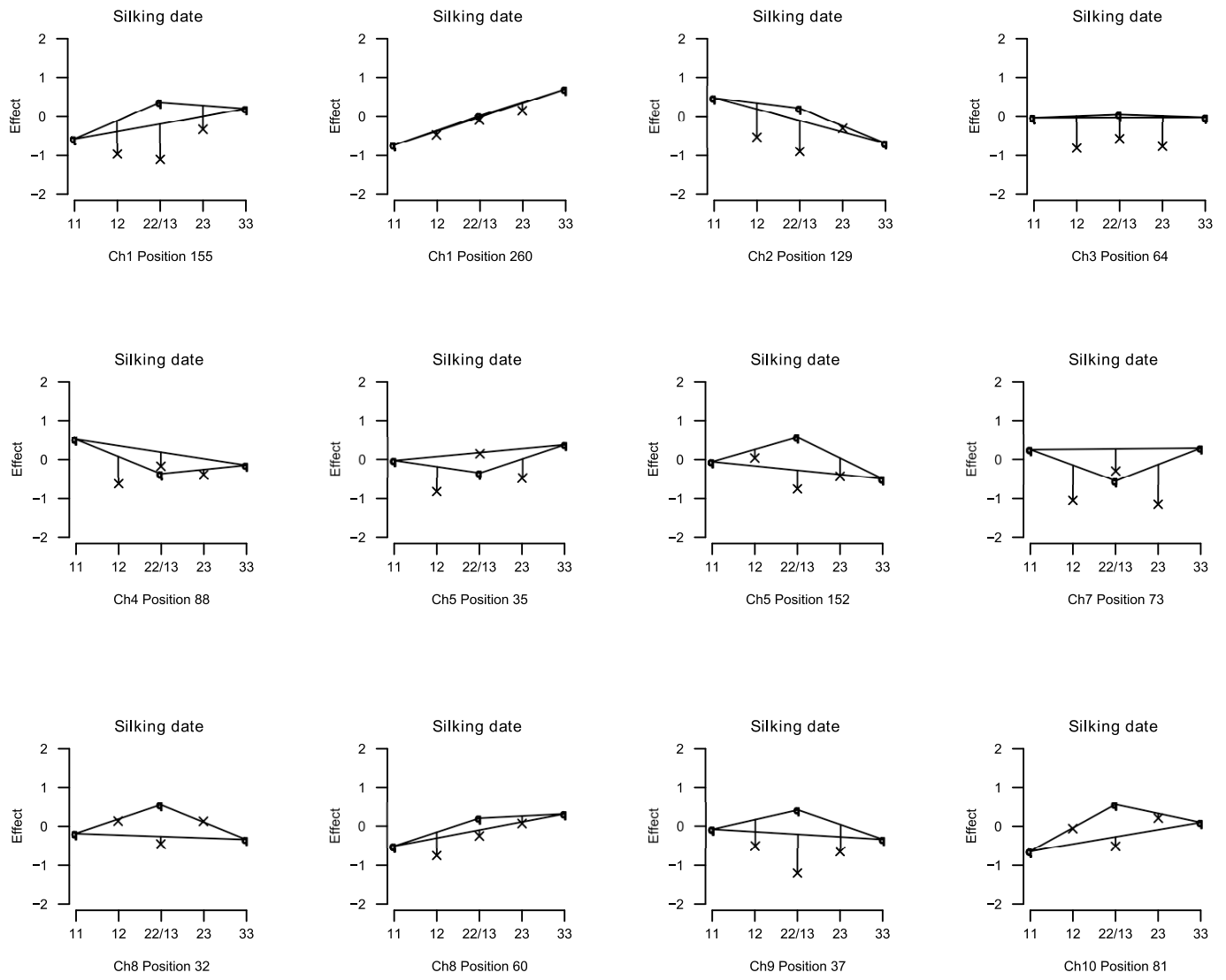


Figure S7 Representation of genetic effects for the QTL detected on the global design for silking date (days)

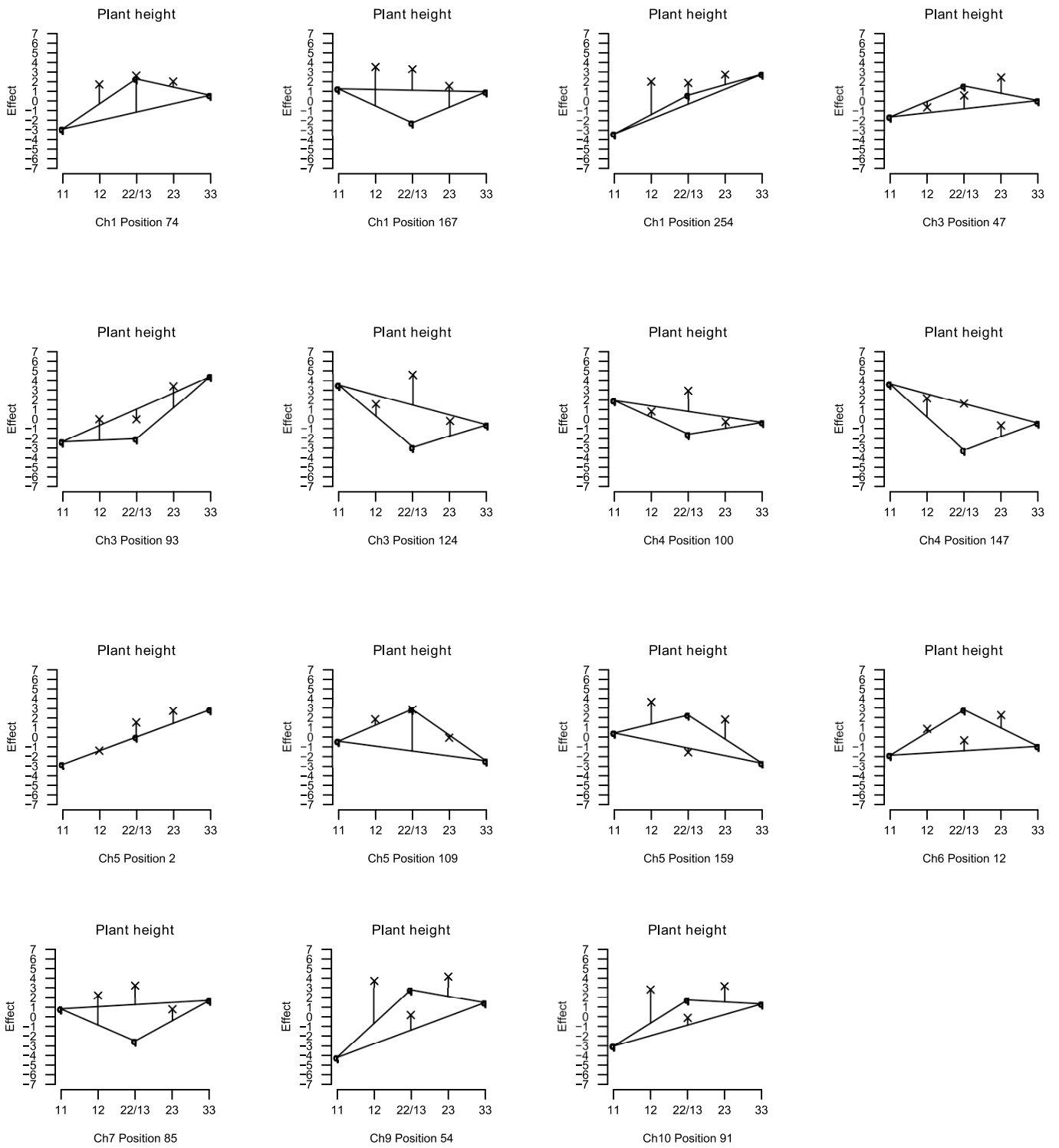


Figure S8 Representation of genetic effects for the QTL detected on the global design for plant height (cm)

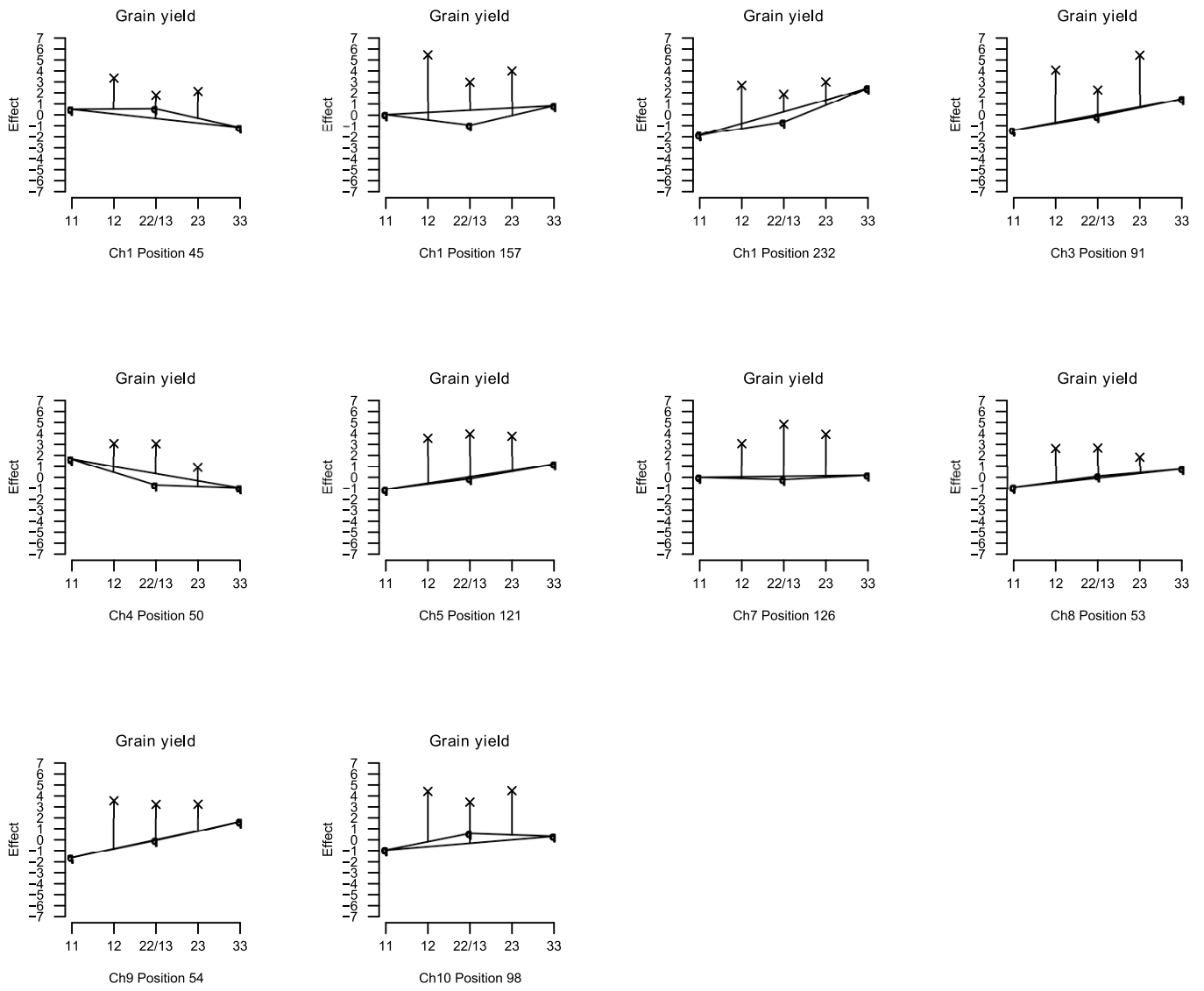


Figure S9 Representation of genetic effects for the QTL detected on the global design for grain yield (q.ha⁻¹)

Table S1 Quantitative trait loci detected for linear transformations Z1 and Z2 for grain moisture at harvest (%), silking date in days from the 1st of January (days), plant height (cm) and grain yield adjusted to zero percent grain moisture ($q\cdot ha^{-1}$)

Traits	Chrom. number	Position (cM)	-log(P) QTL	R ² (%)	CI	Z1						Z2									
						F002F252 pop		F002MBS pop		MBSF252 pop		F002F252 pop		F002MBS pop		MBSF252 pop					
						F002	F252	F002	MBS	MBS	F252	Position (cM)	-log(P) QTL	R ² (%)	CI	F002	F252	F002	MBS	MBS	F252
Grain moisture	1	133	6.63	9.08	126-144	0.08	-0.08	-0.34	0.34	0.29	-0.29	159	5.22	7.11	113-170	0.02	-0.02	0.14	-0.14	-0.16	0.16
	1	258	5.37	7.52	239-269	-0.24	0.24	-0.11	0.11	-0.39	0.39										
	2	105	3.25	4.82	83-114	0.09	-0.09	0.12	-0.12	-0.24	0.24										
	4	79	6.84	9.33	60-96	0.45	-0.45	0.22	-0.22	-0.05	0.05										
	4	202	5.07	7.15	189-206	0.23	-0.23	0.30	-0.30	0.25	-0.25										
	5											77	4.24	5.91	63-107	-0.15	0.15	0.09	-0.09	-0.08	0.08
	6	12	3.89	5.66	5-24	0.01	-0.01	-0.27	0.27	0.19	-0.19										
	8	121	3.96	5.74	55-136	0.08	-0.08	-0.19	0.19	0.26	-0.26										
	9											32	3.68	5.22	9-45	0.03	-0.03	0.14	-0.14	-0.07	0.07
Silking date	1											161	8.64	12.14	152-166	0.24	-0.24	0.25	-0.25	-0.19	0.19
	1	256	4.43	6.48	195-266	-0.29	0.29	-0.36	0.36	-0.21	0.21										
	2											127	4.53	6.85	65-148	0.15	-0.15	0.23	-0.23	-0.08	0.08
	3											69	6.89	9.93	35-77	0.15	-0.15	0.16	-0.16	-0.23	0.23
	4	86	4.46	6.53	76-94	0.45	-0.45	0.08	-0.08	-0.10	0.10										
	5											123	7.24	10.38	109-133	0.26	-0.26	0.07	-0.07	-0.25	0.25
	7											61	11.78	15.94	56-67	0.22	-0.22	0.38	-0.38	-0.20	0.20
9											34	8.96	12.53	24-45	0.28	-0.28	0.19	-0.19	-0.15	0.15	
Plant height	1											153	7.21	10.43	140-160						
	1	254	3.73	5.67	189-206	-1.54	1.54	-1.14	1.14	-1.18	1.18	229	4.73	7.17	212-241	-1.08	1.08	-0.77	0.77	0.90	-0.90
	3	108	6.31	9.04	94-129	-0.31	0.31	1.08	-1.08	-1.89	1.89	124	7.08	10.26	115-134	-1.42	1.42	-0.13	0.13	0.75	-0.75

	4	145	5.11	7.49	88-154	1.28	-1.28	1.83	-1.83	-1.11	1.11										
	5											100	3.04	4.85	31-158	-1.08	1.08	-0.25	0.25	0.30	-0.30
	7											130	9.10	12.81	125-153	-0.90	0.90	-1.18	1.18	1.04	-1.04
	8											91	3.02	4.83	51-143	-1.01	1.01	0.17	-0.17	0.50	-0.50
	9	54	7.43	10.45	48-65	-1.88	1.88	-2.28	2.28	-0.18	0.18	61	3.01	4.82	37-90	-0.60	0.60	-0.56	0.56	0.51	-0.51
Grain	1											153	9.54	12.92	140-159	-1.11	1.11	-1.52	1.52	0.84	-0.84
yield	1	236	4.55	6.33	221-267	-1.02	1.02	-0.39	0.39	-0.97	0.97										
	3											91	7.34	10.25	75-100	0.31	-0.31	-1.31	1.31	1.35	-1.35
	3											189	3.58	5.41	121-201	-0.97	0.97	-0.73	0.73	0.13	-0.13
	5											104	8.41	11.56	96-114	-1.33	1.33	-0.24	0.24	1.27	-1.27
	7											45	4.35	6.44	27-52	-1.04	1.04	-0.13	0.13	0.77	-0.77
	7											126	7.62	10.59	106-157	-0.94	0.94	-0.95	0.95	1.13	-1.13
	8											47	5.20	7.54	36-56	-0.81	0.81	-1.01	1.01	0.44	-0.44
	9	56	3.42	4.91	0-88	-1.16	1.16	-0.31	0.31	-0.43	0.43	25	4.85	7.09	21-57	-0.82	0.82	-1.06	1.06	0.30	-0.30
	10											98	6.16	8.77	41-108	-0.92	0.92	-1.03	1.03	0.89	-0.89

Table S2 Quantitative trait loci detected, significance of additive and dominance effects and epistatic interaction

Traits	QTL number	Chrom. number	Position (cM)	-log(P) QTL	R ² (%)	CI	Additive effect			Dominance effect			Significance of additive effects	Significance of dominance effects	First order additive*additive interaction with QTL	Genetic by background interaction
							MBS	F002	F252	MBS	MBS	F002				
Grain	1	1	139	15.44	7.17	130-142	0.75	-0.39	-0.36	-0.47	-0.15	-0.15	***	ns	5*	
moisture	2	1	260	13.95	6.57	254-268	-0.43	-0.46	0.89	-0.02	-0.17	-0.32	***	ns	8*	
	3	2	105	5.34	2.92	85-115	-0.45	0.34	0.12	-0.16	0.01	-0.29	***	ns	8*	
	4	4	29	5.62	3.05	11-67	-0.28	0.48	-0.20	0.11	-0.08	0.16	***	ns	7*,8**,9**	
	5	4	94	9.53	4.73	78-99	-0.16	0.64	-0.48	-0.01	0.18	0.34	***	ns	1*,12*,13*	
	6	5	134	6.99	3.65	124-140	0.39	0.08	-0.48	-0.31	-0.18	-0.07	***	ns	7**	
	7	6	14	11.14	5.41	9-19	0.67	-0.27	-0.41	-0.05	-0.10	0.09	***	ns	4*,6**	
	8	7	77	7.15	3.72	66-81	-0.42	0.10	0.32	-0.35	-0.31	-0.06	***	ns	2*,3*,4**,9*,11**	
	9	8	59	6.31	3.35	33-68	0.46	-0.51	0.05	-0.07	-0.31	-0.10	***	ns	4**,8*,11***,12***	
	10	8	121	6.92	3.61	93-128	0.44	0.07	-0.51	-0.11	0.39	0.38	***	*	11***,12*	
	11	9	3	4.30	2.45	0-16	-0.28	-0.22	0.50	0.19	0.17	0.12	***	ns	8**,9***,10***,12*	
	12	9	37	12.20	5.85	31-39	0.65	-0.08	-0.57	-0.30	-0.55	-0.03	***	*	5*,9***,10*,11*	
	13	10	77	10.37	5.09	70-86	0.67	-0.51	-0.16	-0.10	-0.08	0.03	***	ns	5*	
	Silking date	1	1	155	8.70	4.58	143-164	0.36	-0.57	0.20	-0.86	-0.60	-0.92	***	***	5*, 12*
2		1	260	7.56	4.07	250-266	0.02	-0.73	0.70	-0.11	-0.21	-0.07	***	ns	-	
3		2	129	7.95	4.24	119-134	0.21	0.48	-0.68	-0.87	-0.06	-0.80	***	***	8*,10*,12*	
4		3	64	2.80	1.84	14-201	0.05	-0.03	-0.02	-0.82	-0.78	-0.55	ns	***	5*,7**	**
5		4	88	4.51	2.66	80-101	-0.37	0.52	-0.15	-0.69	-0.13	-0.36	***	*	1*,4*,6**,7*,8*,10*	
6		5	35	5.61	3.18	10-121	-0.35	-0.03	0.38	-0.63	-0.49	-0.03	***	*	5**,11**	
7		5	152	6.82	3.73	122-158	0.57	-0.06	-0.50	-0.22	-0.47	-0.47	***	ns	4**,5*,8**,9*,12***	
8		7	73	11.55	5.83	61-78	-0.55	0.25	0.29	-0.90	-1.02	-0.57	***	***	3*,5*,7**	
9		8	32	2.81	1.84	0-41	0.54	-0.19	-0.34	-0.05	0.02	-0.19	***	ns	7*	
10		8	60	3.92	2.38	55-74	0.20	-0.51	0.31	-0.59	-0.19	-0.15	***	ns	3*,5*	
11		9	37	9.59	4.97	28-47	0.41	-0.08	-0.34	-0.67	-0.68	-0.99	***	***	6**	
12		10	81	6.24	3.47	61-95	0.55	-0.64	0.09	-0.02	-0.12	-0.24	***	ns	1*,3*,7**	

Plant height	1	1	74	5.17	3.02	50-78	2.28	-2.87	0.60	2.01	0.57	3.74	***	*	5**,6*,10*,12**	
	2	1	167	6.82	3.79	156-176	-2.23	1.27	0.96	4.01	2.19	2.18	***	***	6**,14*	
	3	1	254	6.10	3.45	233-264	0.61	-3.38	2.77	3.38	1.04	2.17	***	*	5**,6*,	
	4	3	47	2.43	1.67	2-78	1.56	-1.62	0.06	-0.61	1.61	1.36	**	ns	9*	
	5	3	93	7.05	3.89	71-111	-2.06	-2.37	4.43	2.20	2.22	-1.06	***	ns	1**,3**,9*,12**,13**,14**,15*	
	6	3	124	8.42	4.52	116-130	-2.93	3.53	-0.61	1.26	1.57	3.12	***	ns	2**,3*,9*,13***,14*,15*	
	7	4	100	4.27	2.59	53-103	-1.59	1.94	-0.35	0.62	0.68	2.13	***	ns	8**,9*,11*	
	8	4	147	9.43	4.97	142-154	-3.23	3.65	-0.42	1.96	1.16	0.00	***	ns	7**,11*	
	9	5	2	8.14	4.39	0-12	0.00	-2.81	2.81	0.02	1.30	1.50	***	ns	4*,5*,6**,7*,11***	
	10	5	109	6.63	3.70	100-115	2.85	-0.42	-2.43	0.61	-0.28	4.26	***	***	1*,12**	*
	11	5	159	9.27	4.90	152-164	2.25	0.42	-2.67	2.30	2.01	-0.43	***	*	7*,8***	
	12	6	12	6.26	3.53	7-19	2.79	-1.85	-0.94	0.37	1.32	1.06	***	ns	1**,5**,6***,10**,14**	*
	13	7	85	6.07	3.44	65-91	-2.50	0.81	1.68	3.04	1.17	1.98	***	*	5**,6**	
	14	9	54	13.86	6.91	47-56	2.77	-4.22	1.44	4.44	2.06	1.56	***	***	2*,5**,6**	
	15	10	91	6.78	3.77	78-99	1.72	-3.03	1.31	3.46	1.65	0.74	***	*	5*,6*	
Grain yield	1	1	45	4.48	2.51	39-53	0.58	0.53	-1.11	2.79	2.39	2.07	**	***	-	
	2	1	157	9.48	4.67	151-164	-0.90	0.05	0.85	5.89	4.02	2.52	ns	***	-	
	3	1	232	9.43	4.65	228-240	-0.62	-1.82	2.44	3.90	2.08	1.58	***	***	-	
	4	3	91	10.35	5.03	83-99	-0.11	-1.37	1.48	4.81	4.75	2.20	***	***	7*	
	5	4	50	6.02	3.19	38-206	-0.70	1.66	-0.96	2.59	1.75	2.70	***	**	-	
	6	5	121	7.58	3.87	98-129	-0.12	-1.10	1.22	4.17	3.19	3.89	*	***	-	
	7	7	126	8.59	4.30	101-131	-0.20	0.00	0.20	3.17	3.92	4.73	ns	***	4*	
	8	8	53	3.60	2.11	46-94	0.12	-0.91	0.79	3.02	1.37	2.73	ns	***	-	
	9	9	54	8.23	4.15	41-65	-0.04	-1.59	1.62	4.38	2.45	3.21	**	***	-	
	10	10	98	9.35	4.62	81-103	0.58	-0.91	0.33	4.58	4.03	3.73	ns	***	-	