Theor Appl Genet (2009) 118:993–1004
DOI 10.1007/s00122-008-0956-3

ORIGINAL PAPER

QTL detection in maize testcross progenies as affected by related and unrelated testers

Elisabetta Frascaroli · Maria Angela Canè · Mario Enrico Pè · Giorgio Pea · Michele Morgante · Pierangelo Landi

Received: 7 May 2008 / Accepted: 20 December 2008 / Published online: 29 January 2009

Abstract The evaluation of recombinant inbred lines (RILs) per se can be biased by inbreeding depression in case of allogamous species. To overcome this drawback, RILs can be evaluated in combination with testers; however, testers can carry dominant alleles at the quantitative trait loci (QTL), thus hampering their detection. This study was conducted on the maize (*Zea mays* L.) population of 142 RILs derived from the single cross B73 × H99 to evaluate the role of different testers in affecting: (1) QTL detection, (2) the estimates of their effects, and (3) the consistency of such estimates across testers. Testcrosses (TCs) were produced by crossing RILs with inbred testers B73 [TC(B)], H99 [TC(H)], and Mo17 [TC(M)]. TCs were field tested in three environments. TC(B) mean was higher than TC(H) mean for all traits, while TC(M) mean was the highest for plant vigor traits and grain yield. As to the number of detected QTL, tester Mo17 was superior to H99 and B73 for traits with prevailing additive effects. Several overlaps among the QTL were detected in two or all the three TC populations with QTL effects being almost always consistent (same sign). For traits with prevailing dominance–overdominance effects, as grain yield, the poor performing tester H99 was clearly the most effective; fewer overlaps were found and some of them were inconsistent (different sign). Epistatic interactions were of minor importance. In conclusion, the three testers proved to affect QTL detection and estimation of their effects, especially for traits showing high dominance levels.

Communicated by C.-C. Schön.

Electronic supplementary material The online version of this article (doi:10.1007/s00122-008-0956-3) contains supplementary material, which is available to authorized users.

E. Frascaroli (✉) · M. A. Canè · P. Landi
Department of Agroenvironmental Sciences and Technologies, University of Bologna, Bologna, Italy
e-mail: elisabetta.frascaroli@unibo.it

M. E. Pè
Sant'Anna School of Advanced Studies, Pisa, Italy

G. Pea
Department of Biomolecular Sciences and Biotechnology, University of Milano, Milan, Italy

M. Morgante
Department of Crop Sciences and Agricultural Engineering, University of Udine, Udine, Italy

Introduction

Populations of recombinant inbred lines (RILs) are valuable materials for genetic studies, such as the detection of quantitative trait loci (QTL), especially because of the advantages associated with the high level of homozygosity and homogeneity of the RILs (Burr and Burr 1991). The high level of RILs homozygosity (close to 100%) brings about estimates of additive genetic variance that are higher than estimates obtained in many other populations (e.g., the additive genetic variance in a RILs population is expected to be twice as great as that of a population represented by $F_{2:3}$ lines). In turn, this higher genetic variance should lead to a higher heritability and, thereby, to a more powerful QTL detection (Lande and Thompson 1990). This was the case in the study of Austin et al. (2000, 2001), who detected more QTL in the $F_{6:8}$ population than in the $F_{2:3}$ population

derived from the same source. Moreover, the high level of genetic homogeneity within each RIL minimizes the problems related to the presence of genetic variation; hence, RILs can be repeatedly reproduced avoiding troubles associated with changes of allelic frequencies (mainly due to unintentional selection and to genetic drift) which can occur when reproducing heterogeneous lines. As compared to the populations of doubled haploids, directly produced by the source F_1, populations of RILs should also allow a higher mapping resolution because of the greater opportunity for recombination during the process of RILs' development.

However, for cross-pollinated species like maize (*Zea mays* L.), the evaluation of RILs per se can lead to results biased by inbreeding depression, especially for traits largely affected by dominance. To overcome such a drawback, the genetic analyses can be conducted by testing the RILs in hybrid combination, i.e., crossed with testers. The choice of testers is a crucial issue because they can affect the genetic variance among testcrosses (TCs) (Hallauer and Miranda 1988) and, hence, the power of QTL detection. An interesting approach, that recalls aspects of applied plant breeding, could be to choose elite inbred lines to which the best RILs will be eventually crossed to produce commercial hybrids. In case of maize, successful hybrids are often represented by crosses between inbred lines originated from Reid Yellow Dent germplasm, especially Iowa Stiff Stalk Synthetics (BSSS), and lines of different origin, especially Lancaster Sure Crop (LSC) germplasm (Hallauer 1990); hence, a proper tester for a RIL population originated from one gene pool could be chosen from the complementary one. However, the tester choice could be more problematic in case of a RIL population originated from a cross between inbred lines belonging to the two germplasms. The utilization of elite inbred lines can also bring about drawbacks associated with masking effects exerted by the dominant alleles carried by the testers, so that the ability of QTL detection can largely depend on the testers' genotypes (Lübberstedt et al. 1997; Austin et al. 2000; Ajmone Marsan et al. 2001). Therefore, an important aspect concerning QTL analysis in testcross progenies is the consistency of QTL effects across testers (Melchinger et al. 1998). The drawbacks in QTL analysis arising from the confounding effects due to the different alleles brought in by the testers could be at least partly overcome using as testers the parental inbreds of the RILs' population. This study was conducted on a maize population of RILs crossed with three inbred lines, i.e., the two parents of the source single cross and an unrelated inbred; the objectives were to evaluate the role played by these three testers with respect to (1) the detection of QTL, (2) the estimates of their effects, and (3) the consistency of such estimates across testers.

Materials and methods

Plant materials

A population of 142 RILs was developed following the single seed descent procedure for 12 selfing generations ($F_{12:13}$); the source material was the single cross B73 × H99. B73 and H99 are inbred lines selected from BSSS and from Illinois Synthetic 60C, respectively, and largely differ for both agronomic (Frova et al. 1999; Frascaroli et al. 2007) and molecular characteristics (Livini et al. 1992; Lu and Bernardo 2001). TCs were produced by crossing the 142 RILs with the parental inbred lines B73 [TC(B)] and H99 [TC(H)], and with the unrelated inbred line Mo17 [TC(M)]. Inbred line Mo17 was derived from the cross 187-2 × C103 and can be assigned to the same heterotic group as H99 (i.e., LSC or, more loosely, non-BSSS), despite H99 and Mo17 have diverse origin and markedly differ for agronomic (Austin et al. 2000, 2001) as well as molecular characteristics (Livini et al. 1992; Lu and Bernardo 2001). In order to have control hybrids, the unrelated tester Mo17 was also crossed to the two parental inbreds B73 and H99.

Field experiments and data analysis

The three populations of TC progenies were field tested in a larger study including also other materials (i.e., RILs per se and TCs with the source F_1) as described in detail by Frascaroli et al. (2007). Briefly, the field trials were carried out in 2002 at three locations in Northern Italy (Bologna, Cremona, and Milan). In each location, the field layout was a modified split-plot design with two replications; the three testers were the main plots and the RILs (combined with the tester) were the subplots. The population of TC(M) progenies also included, as controls, the single crosses B73 × Mo17 and H99 × Mo17, which were entered three times in each replication. Plots were single rows 4.40 m long, 0.80 m wide, and included (after thinning) 22 plants at a density of 6.25 plants m^{-2}. Main plots were separated by two border rows due to the different levels of plant vigor expected for the three populations of TCs. The usual field techniques for maize were followed to achieve favorable growing conditions (in particular, three to four irrigations were made in each trial).

The following traits were measured at the single-plot level in each trial: (1) days to pollen shedding (PS), as interval between sowing date and PS date (assessed when 50% of plants per plot had extruded at least nine anthers); (2) plant height (PH) measured at the flag leaf collar on three competitive plants per plot; (3) kernel moisture (KM) at harvest; (4) grain yield (GY) adjusted to 15.5% moisture; (5) kernel weight (KW) adjusted to 15.5% moisture on a

sample of 100 kernels; and (6) number of kernels per plant (NK), as ratio between GY per plant and KW.

The analysis of variance (ANOVA) was conducted in accordance with the modified split-plot design for each environment (location) and then was combined across environments. A mixed model was adopted, considering environments and RILs as random and testers as fixed variables. Because of the high significance of tester × RIL interaction, the ANOVA was also conducted separately for each TC population. Analyses were conducted using SAS GLM and VARCOMP procedures (SAS Institute 1996), and least square means over locations were used for subsequent analyses.

For each TC population, the estimates of variance components for RILs (or genotypes, σ_g^2), genotype × environment (σ_{ge}^2), and error (σ^2) were computed from expectations of mean squares. Standard errors of these variance components were calculated according to Hallauer and Miranda (1988, p. 49). The heritability (h^2) values were calculated across the three environments (n) and the two replications (r) per environment, as $h^2 = \sigma_g^2/(\sigma_g^2 + \sigma_{ge}^2/n + \sigma^2/nr)$. The h^2 confidence intervals were calculated according to Knapp et al. (1985).

For the same trait, the relationship between the performances of TC pair [i.e., TC(B) vs. TC(H), TC(B) vs. TC(M), and TC(H) vs. TC(M)] was investigated by simple phenotypic correlations, using the mean values of each TC across replications and environments.

Genetic linkage map

The linkage map is an updated version of the map employed in Frascaroli et al. (2007) and comprises 207 markers spanning a total of 2,351.8 cM (Kosambi mapping function). In particular, the new map includes several additional public SSR (available at http://www.maizegdb.org) as well as AFLP and molecular markers developed from maize expressed sequence tentative contigs. The updated map can be found in the supplementary material (Fig. S1).

Analysis of main-effect QTL

For each trait and TC population, the analysis of main-effect QTL was conducted on mean values across environments. Composite interval mapping (CIM) (Zeng 1994) was used to identify QTL by means of PLABQTL software (Utz and Melchinger 1996). Details concerning choice of cofactors were the same as in Frascaroli et al. (2007). LOD (=0.217 LR) threshold for declaring a putative QTL for each trait and TC was defined by 1,000 permutations (Churchill and Doerge 1994). QTL detected with different TC or 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 estimated by the adjusted coefficient of determination of regression (R^2_{Adj}) fitting a model that includes all detected QTL.

Following Melchinger et al. (1998) and Blanc et al. (2006), the model underlying TC progenies with a given tester can be written as:

$$Y_{it} = m_{\text{B73}} + s^q x_i^q + \Sigma_k s_k^c x_{ki}^c + e_{it}$$

where Y_{it} is the phenotypic value of the TC progeny of RIL i with tester t; m_{B73} is the mean of TC progenies carrying, at the putative QTL q, the B73 allele provided by the RILs; s^q (s^c) is the estimated effect of substituting the B73 allele (B) with the H99 allele (H) at the QTL q (or at kth cofactor c) in combination with tester t; x_i^q is the conditional expectation of a dummy variable that assumes value 0 or 1 if the genotype of the RIL i at the QTL q corresponds to the parent B73 or H99, respectively, given the observed genotypes at the flanking markers; x_i^c is a dummy variable that assumes value 0 or 1 if the genotype of the RIL i at the kth cofactor c corresponds to the parent B73 or H99, respectively; e_{it} is the residual error.

For the putative QTL, additive genetic effects are expected to be $-a$ and a in the homozygotes for B and H alleles, respectively, and dominant genetic effect is expected to be d in the heterozygote. Therefore, in case of TC(B) the effect of a putative QTL (s^q) is expected to be $a + d$, being estimated from the difference between the heterozygote and the homozygote for B allele. In case of TC(H), the putative QTL effect (s^q) is expected to be equal to a–d, being estimated from the difference between the homozygote for H allele and the heterozygote. In case of TC(M), s^q is the difference between the dominance effect displayed by the H99/Mo17 allelic combination and that displayed by B73/Mo17 allelic combination.

Epistatic QTL analysis

A mixed linear model was used to first confirm main-effect QTL found in the previous analysis and then to map digenic epistatic QTL. The analysis was conducted with QTLMapper (Wang et al. 1999) first without epistasis and then including epistasis in the model. A significance threshold of $P \leq 0.001$ and $R^2 > 5\%$ was adopted to declare an epistatic QTL (Wang et al. 1999; Li et al. 2001). Effects and statistics tests associated with significant main-effect and epistatic QTL were obtained with the restricted maximum-likelihood (REML) estimation method, as described by Wang et al. (1999). For the most interesting interactions, means of genotypic classes corresponding to the two pairs of allelic combinations (two parental and two recombinant classes) of the interacting QTL were obtained. To this purpose, only TC progenies corresponding to RILs with the flanking

markers of each interacting QTL having the same phase, i.e., coming from the same parent (neglecting double crossovers), were taken into account.

Results

Performances of TC populations

The ANOVA (not shown) revealed that differences among environments (locations) were significant for all traits (at $P \leq 0.05$ or $P \leq 0.01$) except PH, thus indicating that TCs were investigated across an appreciable range of growing conditions. In particular, for GY the location mean ranged from 6.05 Mg ha^{-1} in Cremona to 9.52 Mg ha^{-1} in Milan, indicating that medium to high yield levels were attained. The RIL × environment interaction, even when significant, was always much smaller than the mean square due to RILs; therefore, the analyses were conducted across the three environments. The tester × RIL interaction was highly significant for all traits, indicating that the relative hybrid performance of the RILs markedly varied depending on the inbred line used as tester. For this reason, the analysis of RILs' performance was conducted separately for each tester.

The mean value of TC(B) was for all traits higher than the mean value of TC(H) (Table 1), whereas the mean value of TC(M) was higher than that of the other two TCs for PH, GY, KW, and NK. The estimate of σ^2_g was highly significant for all traits and so was the estimate of σ^2_{ge}, with the only exception of PH for TC(H) and of KM for TC(B). The estimate of σ^2_g was the highest in TC(H) for all traits except KM, whereas it was often the lowest in TC(M). The estimate of σ^2_{ge} was always smaller than σ^2_g, except for GY and NK in TC(M). The heritability followed a trend similar to that of σ^2_g.

In the trial of TC(M) progenies, the B73 × Mo17 control was superior to the H99 × Mo17 control for all traits (data not shown); in particular, for GY their mean values were 10.68 and 7.64 Mg ha^{-1}, respectively (Fig. 1). The GY mean value of these two controls was significantly superior ($P \leq 0.01$) to the mean value of TC(M) (9.16 and 8.54 Mg ha^{-1}, respectively). Despite the large GY difference between the two checks, several TC(M) progenies yielded less than the low-yielding H99 × Mo17 check, while three progenies yielded more than the high-yielding B73 × Mo17 check, suggesting that the increasing alleles were not all provided by the better parent B73. As to the other traits (data not shown), the comparison between the mean values of the two checks and of the TC(M) progenies was not significant; moreover, the range in TC(M) performance markedly transgressed the two checks with the

Table 1 Estimates of means, variance components, and heritability of maize testcross (TC) progenies of 142 RILs (from single cross B73 × H99) crossed with inbred tester B73 [TC(B)], H99 [TC(H)], and Mo17 [TC(M)] for PS (pollen shedding, as interval from sowing), PH (plant height), KM (kernel moisture), GY (grain yield), KW (kernel weight), and NK (number of kernels per plant)

Trait and parameter	TC(B)	TC(H)	TC(M)
PS			
Mean (days)	74.0	70.7	70.7
σ^2_g	0.85 ± 0.21	1.21 ± 0.19	1.13 ± 0.17
σ^2_{ge}	0.75 ± 0.14	0.32 ± 0.10	0.29 ± 0.09
σ^2	1.64 ± 0.11	1.50 ± 0.10	1.37 ± 0.09
h^2 [a]	0.62	0.77	0.78
90% CI on h^2 [b]	0.52–0.69	0.71–0.82	0.72–0.82
PH			
Mean (cm)	206	147	234
σ^2_g	91.0 ± 13.4	170.3 ± 21.9	47.0 ± 9.1
σ^2_{ge}	15.3 ± 6.2	2.7 (NS)	38.4 ± 7.4
σ^2	85.1 ± 6.5	87.9 ± 6.0	85.9 ± 5.9
h^2	0.83	0.92	0.63
90% CI on h^2	0.78–0.86	0.89–0.93	0.54–0.71
KM			
Mean (%)	28.8	26.5	27.0
σ^2_g	2.47 ± 0.35	1.33 ± 0.23	1.44 ± 0.22
σ^2_{ge}	0.21 (NS)	0.68 ± 0.16	0.38 ± 0.11
σ^2	2.80 ± 0.19	2.14 ± 0.15	1.57 ± 0.11
h^2	0.82	0.70	0.79
90% CI on h^2	0.78–0.86	0.62–0.75	0.73–0.83
GY			
Mean (Mg ha^{-1})	7.50	6.33	8.54
σ^2_g	1.04 ± 0.17	1.30 ± 0.18	0.49 ± 0.13
σ^2_{ge}	0.43 ± 0.09	0.24 ± 0.07	0.83 ± 0.16
σ^2	1.15 ± 0.08	0.99 ± 0.07	1.76 ± 0.12
h^2	0.76	0.84	0.46
90% CI on h^2	0.69–0.80	0.80–0.87	0.32–0.57
KW			
Mean (mg)	321	307	340
σ^2_g	208 ± 58	416 ± 59	364 ± 54
σ^2_{ge}	150 ± 24	93 ± 21	164 ± 23
σ^2	232 ± 16	270 ± 19	206 ± 14
h^2	0.70	0.85	0.80
90% CI on h^2	0.62–0.76	0.81–0.88	0.75–0.84
NK			
Mean (no.)	371	328	402
σ^2_g	2,196 ± 363	2,427 ± 357	706 ± 249
σ^2_{ge}	942 ± 230	318 ± 171	1,589 ± 337
σ^2	3,018 ± 207	2,768 ± 190	4,112 ± 282
h^2	0.73	0.81	0.37
90% CI on h^2	0.66–0.78	0.76–0.85	0.21–0.49

NS not significant

[a] On a mean basis across three environments and two replications per environment

[b] Confidence intervals (CI) calculated according to Knapp et al. (1985)

only exception of PH. These results thus suggest that for PH there was mainly an association of the increasing alleles in one parent (i.e., B73), while for the other traits the increasing alleles were dispersed in the two parents.

The correlation analysis between the performances of TC populations (Table 2) showed the highest coefficients for PS, PH, KM, and KW, especially in case of TC(H) versus TC(M). In contrast, the lowest correlation coefficients were obtained for GY and NK; in particular, the coefficients for these two traits were negative in TC(B) versus TC(H), though not significantly different from zero.

Table 2 Phenotypic correlations for each trait between pair of TC populations of 142 RILs crossed with inbred tester B73 [TC(B)], H99 [TC(H)], and Mo17 [TC(M)] investigated across three environments

Trait	TC(B) vs. TC(H)	TC(B) vs. TC(M)	TC(H) vs. TC(M)
PS	0.46**	0.54**	0.65**
PH	0.41**	0.51**	0.69**
KM	0.54**	0.67**	0.56**
GY	−0.13 (NS)	0.17*	0.41**
KW	0.51**	0.69**	0.67**
NK	−0.08 (NS)	0.14 (NS)	0.30**

NS not significant

*, ** Significant at $P \leq 0.05$ and $P \leq 0.01$, respectively

Main-effect QTL

QTL analysis was conducted on mean values across environments and separately for each tester (Table 3). For PS, the effects of the detected QTL were negative in most cases and the highest LOD score (18.8) was observed in TC(B) for a QTL accounting for 34.6% of phenotypic variance (σ_p^2); the R^2 obtained with the simultaneous fit of all detected QTL was maximum in TC(M) (39.4%). Considering PH, QTL effects were in most cases negative in TC(H), always negative in TC(M), whereas there was a balance between negative and positive effects in TC(B). Both the highest LOD (19.1) for a single QTL ($R^2 = 27.1\%$) and the highest R^2 of the model including all QTL (59.3%) were observed in TC(H). For KM, QTL effects were always negative in TC(M), whereas there was some balance between positive and negative effects in TC(B) and in TC(H). The highest LOD (10.0) was detected in TC(B) ($R^2 = 10.9\%$), while the highest R^2 of the model including all QTL (34.5%) was observed in TC(M). The analysis of GY identified seven QTL in TC(B), 11 in TC(H), 6 in TC(M), and 18 on the whole, with effects ranging from −0.98 to 0.89 Mg ha^{-1}. These effects were almost always positive in TC(B), almost always negative in TC(H), whereas there was the same number of negative and positive effects in TC(M). The QTL with the highest LOD (11.9) was mapped in TC(B) ($R^2 = 12.4$). When considering all detected QTL, R^2 was 25.1% in TC(B), 56.5% in TC(H), and 32.2% in TC(M). For KW, QTL effects were mainly negative, especially in TC(H) and TC(M). The highest LOD (13.2) was estimated in TC(H) and the highest R^2 of the model including all QTL (65.7%) was in TC(M). As to NK, QTL effects were always positive in TC(B) and always negative in TC(H); both the highest LOD score (9.3) for a single QTL and the highest R^2 of the model including all QTL were found in TC(H).

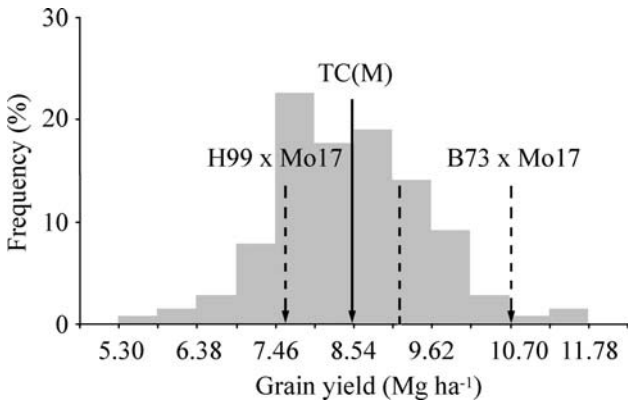

Fig. 1 Frequency distribution for GY of TC(M) population in comparison with check hybrids B73 × Mo17 and H99 × Mo17 (*dashed arrows*); the *dashed line* indicates the mean of the two check hybrids. The highest *continuous arrow* indicates the mean of the 142 TC(M) progenies. The class interval is equal to the standard error from the combined analysis of the three TC(M) trials

In summarizing across traits the findings reported in Table 3, 31 QTL were detected in TC(B), 43 in TC(H), 40 in TC(M), and 74 on the whole. In addition, the effects of these QTL were more frequently positive in TC(B) (19 out of 31), whereas they were more frequently negative in TC(H) (37 out of 43) and in TC(M) (28 out of 40).

Based on the data presented in Table 3, it can also be noted that 42 QTL were detected in only one TC, whereas the other 32 were common to at least two TCs. As eight of these 32 QTL were common to all the three TCs, the whole number of overlaps was 48, obtained by summing 24 overlaps between two TCs and 24 overlaps concerning the eight QTL common to all three TCs. As reported in Table 4, 44 of these overlaps (corresponding to 92%) were consistent, i.e., had effects of the same sign. The whole number of overlaps across traits was 12 for TC(B) versus TC(H), 15 for TC(B) versus TC(M) and 21 TC(H) versus TC(M). When overlaps were considered across TCs, the highest number was observed for KW (20) with most of these overlaps showing consistent effects. Eleven

Table 3 Parameters of putative main-effect QTL estimated from TC populations of 142 RILs crossed with inbred tester B73 [TC(B)], H99 [TC(H)], and Mo17 [TC(M)] for the traits investigated across three environments

Trait and bin	Flanking markers	TC(B)			TC(H)			TC(M)		
		LOD	R^2 [a]	s[b]	LOD	R^2	s	LOD	R^2	s
PS (days)										
1.07	*bnlg1025–dupssr12*				4.2	5.3	−0.5	3.9	8.4	−0.6
2.02	*bnlg1092–E38M6109*	4.9	6.5	−0.8						
7.02	*bnlg2203–phi008b*				11.0	19.8	1.3	5.7	4.4	0.5
8.03	*umc1904–bnl9.08*	18.8	34.6	−1.8				3.6	10.1	−0.8
8.05	*phi121–bnlg666*				11.1	25.5	−1.3	15.2	22.7	−1.2
Total[c]			35.0	2.6		33.8	3.1		39.4	3.1
PH (cm)										
1.04	*dupssr26–E35M4910*	6.4	11.8	6.5						
1.07	*bnlg1025–dupssr12*	12.4	11.1	−6.4	19.1	27.1	−12.2	3.7	9.7	−5.4
2.04	*bnlg125–dupssr27*	6.0	3.4	3.4						
2.08	*mmc0271–bnlg198*	10.2	10.8	−6.5	6.5	14.9	−8.2	8.6	18.3	−7.8
2.09	*csu64a–bnlg469b*	8.2	5.6	−4.3	5.6	8.2	−5.7			
3.02	*bnlg1325–umc2071*				4.5	6.4	−5.2	7.4	12.5	−6.1
3.04	*umc42b–bnlg602*				7.3	0.5	−1.6			
3.05	*bnlg420–bnlg1505*	8.0	4.7	3.8						
3.06	*dupssr23–umc1730*				6.4	5.7	−5.6			
4.10	*umc1503–bnlg589*	8.8	6.4	4.6						
5.05	*TC315883–bnl5.40*							4.9	11.6	−6.4
7.04	*umc1029–umc1944*				5.6	3.3	3.5			
8.01	*umc1075–AZM4_59852*				*3.2*	*6.0*	*−5.0*	6.0	9.7	−5.3
8.03	*umc1904–bnl9.08*	5.6	1.2	−2.1	7.0	4.0	−4.7			
8.05	*bnlg666–bnlg162*				4.0	8.9	−7.7	6.6	26.0	−10.5
10.03	*bnlg1451–umc1962*	7.6	9.8	7.0						
Total			29.5	44.7		59.3	59.4		47.5	41.5
KM (%)										
1.07	*bnlg1556–bnlg1025*							7.4	19.1	−1.2
2.08	*TC326717–dupssr25*	4.8	13.3	1.2	*2.4*	*7.6*	*0.8*			
2.09	*csu64a–bnlg469b*	10.0	10.9	1.2						
5.03	*bnlg105–bnlg557*				4.7	7.3	−0.9	3.9	12.5	−0.9
8.03	*umc1904–bnl9.08*	5.1	9.9	−1.0				3.4	9.0	−0.8
8.05	*phi121–bnlg666*							5.0	2.9	−0.4
9.07	*bnlg619–umc1137*							5.5	7.5	−0.7
Total			30.2	3.4		14.9	1.7		34.5	4.0
GY (Mg ha^{-1})										
1.04	*bnlg2295–dupssr26*	5.5	6.4	0.60						
1.06	*TC344182–umc1035*	3.6	5.0	0.66	3.5	2.0	−0.31			
2.04	*bnlg381–phi083*							4.3	2.8	0.36
4.03	*umc2176–umc1963*				4.8	5.0	−0.39			
5.04	*dupssr10–dupssr7*				7.9	7.8	−0.47	6.0	10.5	−0.66
6.01	*phi075–bnlg1371*	11.9	12.4	0.89				9.2	4.5	0.49
6.07	*phi070–TC315926*	6.0	5.6	0.50						
7.02	*phi034–bnlg398*				4.9	1.5	0.20			
7.03	*umc1015–bnlg434*	5.3	10.2	0.72						
7.04	*umc1251–umc1029*							7.9	4.9	0.53
8.01	*umc1075–AZM4_59852*				5.3	9.8	−0.54			

Table 3 continued

Trait and bin	Flanking markers	TC(B)			TC(H)			TC(M)		
		LOD	R^2 [a]	s[b]	LOD	R^2	s	LOD	R^2	s
8.03	*bnl9.08–phi121*	3.7	3.5	0.70				*2.6*	*1.4*	*−0.42*
8.05	*phi121–bnlg666*	4.9	4.6	−0.80	8.9	8.3	−0.98	*2.3*	*3.6*	*−0.69*
8.08	*umc1268–E35M4901*				5.6	7.8	−0.51			
9.04	*bnlg430–bnlg1209*				6.3	16.6	−0.92			
9.07	*umc1137–bnlg1129*				4.1	6.8	−0.43			
10.03	*bnlg1451–umc1962*				5.9	7.1	−0.67			
10.04	*umc2003–TC334690*				6.2	8.0	−0.56			
Total[c]			25.1	4.87		56.5	5.98		32.2	3.15
KW (mg)										
1.04	*bnlg2295–dupssr26*	5.2	13.7	14.2				7.0	9.9	11.5
1.08	*dupssr12–TC316139*							6.7	16.6	−15.4
2.04–2.07	*E38M6101–E35M4908*				3.6	3.4	−7.5	5.8	9.3	12.0
2.08	*mmc0271–bnlg198*				6.9	4.6	−8.4	8.5	27.4	−24.7
2.09	*dupssr25–bnlg1520*							7.4	20.1	18.6
3.04	*phi029–bnlg1638*	4.9	11.8	13.4						
3.05	*bnlg420–bnlg1505*							10.8	23.2	18.4
4.07	*bnlg1621a–dupssr34*				8.3	3.1	−8.8			
4.08	*E38M6104–phi093*	4.2	12.3	14.5	3.4	7.8	13.3	7.2	14.7	14.0
5.03	*bnlg105–bnlg557*	5.0	9.7	−11.5	5.4	18.8	−17.1	6.1	17.9	−16.4
5.05	*TC315883–bnl5.40*				13.2	14.7	−16.9	*3.1*	*9.5*	*−13.5*
5.08	*umc1792–php20523b*				6.5	2.9	6.1			
8.01	*umc1075–AZM4_59852*	*2.2*	*6.0*	*−9.0*	*2.0*	*7.9*	*−9.8*	5.5	13.5	−14.1
8.03	*E38M6107–umc1904*	3.7	5.6	−9.6	4.8	1.9	−5.3	3.6	7.0	−9.8
8.05	*phi121–bnlg666*	4.3	7.2	−12.5	7.8	6.0	−10.7	6.9	18.7	−18.6
10.03	*umc1962–umc2016*				4.9	5.0	−7.6	*3.5*	*8.8*	*−10.2*
Total			37.4	84.8		43.0	111.5		65.7	197.2
NK (no.)										
1.06	*TC344182–umc1035*				4.1	6.2	−28	*2.7*	*2.1*	*−12*
2.04	*bnlg381–phi083*							3.6	1.3	−9
4.03	*nc135–umc2176*				9.3	12.0	−55			
5.04	*dupssr10–dupssr7*				5.2	6.7	−26			
6.01	*phi075–bnlg1371*	5.5	14.0	43				8.2	13.8	27
7.03	*umc1408–umc1134*	4.8	9.8	32						
7.04	*umc1251–umc1029*							3.4	1.4	8
8.02	*bnlg1194–umc1304*				5.9	7.6	−28			
8.03	*E38M6107–umc1904*	6.1	8.5	30				*3.0*	*7.0*	*−22*
8.07	*umc1149–npi268a*				7.3	3.3	−19			
10.04	*umc2003–TC334690*				6.4	11.4	−36			
10.07	*umc1196–bnlg1360*							3.6	2.8	13
Total			22.5	104		29.9	192		16.1	91

Values in roman typeface: "significant QTL", i.e., with LOD reaching the threshold for $P \leq 0.10$ determined by 1,000 permutations (threshold corresponded to a minimum value of LOD = 3.3)

Values in italics: "suggested QTL", i.e., with LOD reaching the threshold for $P \leq 0.30$ (minimum LOD = 2.0) and mapping approximately at the same position in more than one TC

[a] Proportion (%) of phenotypic variance explained by the QTL

[b] Average effect of substituting the allele of B73 with the allele of H99, see "Materials and methods" for genetic effects

[c] Proportion (%) of the total phenotypic variance obtained by simultaneous fit of all putative QTL for the trait and sum of the *s*-effect absolute values

Table 4 Number of overlaps between putative QTL common to at least a pair of TC populations and consistency of their effects for each trait

Trait	TC(B) vs. TC(H)		TC(B) vs. TC(M)		TC(H) vs. TC(M)		Total	
	c[a]	i[a]	c	i	c	i	c	i
PS	0	0	1	0	3	0	4	0
PH	4	0	2	0	5	0	11	0
KM	1	0	1	0	1	0	3	0
GY	1	1	2	1	2	0	5	2
KW	5	0	6	0	8	1	19	1
NK	0	0	1	1	1	0	2	1
Total	11	1	13	2	20	1	44	4

[a] c and i are common QTL with the same sign (consistent) or different sign (inconsistent), respectively

overlaps were noted for PH and all of them were consistent. Fewer overlaps were found for PS, KM, GY, and NK; only for the former two traits, overlaps were always consistent.

Epistatic QTL

Most of main-effect QTL detected with PLABQTL were also detected with QTLMapper when epistasis was not included in the model and QTL effect estimates were similar with the two methods; the few QTL not in common were those with the lowest R^2 values (data not shown). The analysis of epistasis conducted with QTLMapper led to the detection of 29 interactions across all the investigated traits (Table 5). The epistatic effects were in many instances smaller than the main effects previously seen in Table 3. Across testers, interactions varied from a minimum of two for NK and three for GY to a maximum of seven for PS. For GY, in particular, one interaction was detected in TC(B) and two interactions in TC(M). To have a better insight into these two interactions detected in TC(M), the mean values of the two parental and of the two recombinant genotypic classes were taken into account (Fig. 2). Only those progenies with markers flanking each epistatic interval coming from the same parent were considered (see "Materials and methods"). The number of sampled progenies was on average about 20 per genotypic class; it follows that the information given by the corresponding mean values should be considered just as an indication of the type of epistatic interactions being analyzed. Despite this limitation, mean values provided a clear trend, as the two parental classes (and especially the one recalling B73) exceeded the two recombinant classes, thus accounting for the positive sign of both interactions.

Most of the detected interactions involved marker intervals with non-significant main effects; the exceptions concerned four interactions for PS, two for PH and KM, and one for KW and NK. Across traits, ten interactions were detected in TC(B), 11 in TC(H), and 8 in TC(M); the positive interactions were three for TC(B) and six for both TC(H) and TC(M), corresponding to 30% [(TC(B)], 55% [(TC(H)], and 75% [(TC(M)] of the interactions detected for each of the three TCs.

Discussion

Performances of TC progenies

The higher mean value exhibited by TC(B) over TC(H) for all investigated traits was consistent with the well-known greater lateness, size and productivity of B73 as compared with H99 (Frova et al. 1999; Frascaroli et al. 2007). The superiority of TC(M) over both TC(B) and TC(H) for traits related to plant vigor and yield can be at least partly explained as the TCs with the unrelated tester Mo17 are expected to have an inbreeding coefficient (F) close to zero. In contrast, the F value of both TC(B) and TC(H) is expected to be, on average, equal to 0.5 and, hence, inbreeding depression can reduce their performances for traits with important dominance effects.

The highest values of σ_g^2 and of h^2 observed with the low performing tester H99 for most traits is consistent with the genetic theory (Hallauer and Miranda 1988, p. 271), as this tester is likely homozygous for the unfavorable recessive allele at several loci and, hence, is expected to reveal a greater genetic variation among TCs, especially for traits with a high level of dominance.

For GY, the mean of check hybrids (i.e., the cross of each parental inbred and Mo17) exceeded the mean of TC(M) with a difference that should mainly represent the net effect of epistasis across loci. In fact, the average allelic frequencies of check hybrids are expected to be substantially the same as those of TC(M), whereas differences should be found for genotypic frequencies between the checks (involving crosses with parental genotypes only) and TC(M) (involving crosses with both parental and recombinant genotypes). Therefore, the lower GY value observed in the TC(M) can be ascribed to the dissipation (as a result of recombination) of those favorable allelic combinations previously accumulated in the two parents and determining positive epistatic interactions with tester Mo17. As to the other traits, the lack of significant differences between the check hybrids' mean and TC(M) mean suggested either the lack of important epistatic interactions or the presence of interactions of opposite sign canceling each other.

Table 5 Epistatic QTL detected in the three TC populations of 142 RILs crossed with inbred tester B73 [TC(B)], H99 [TC(H)], and Mo17 [TC(M)] for the traits investigated across three environments

Trait and TC population	Bin	Marker interval *i*	Bin	Marker interval *j*	LOD	ss_{ij}[a]
PS (days)						
TC(B)	3.04	*bnlg1638–bnlg1019a*	5.02	*umc1587–bnlg105*	4.5	−0.5
	8.05	***phi121–bnlg666***	10.07	*dupssr37–umc1196*	5.3	−0.5
TC(H)	1.04	*bnlg2295–dupssr26*	7.05	*umc1295–php20690a*	7.3	−0.5
	1.08	***dupssr12–TC316139***	7.02	*umc1986–phi034*	5.8	0.3
	5.05	*dupssr7–bnl5.71a*	6.06	*phi070–TC315926*	4.4	−0.4
TC(M)	1.08	***bnlg1025–dupssr12***	7.02	***phi008b–bnlg657***	3.8	0.4
	5.04–5.05	*dupssr10–dupssr7*	8.05	***phi121–bnlg666***	21.3	0.4
PH (cm)						
TC(B)	2.07	*TC333426–phi127*	2.08	*TC326717–dupssr25*	4.2	−3.5
	7.02	*E35M5507–umc1986*	10.02	***php200075a–bnlg1451***	5.2	−4.0
TC(H)	1.08	***dupssr12–TC316139***	4.1	*umc1101–umc1503*	14.9	3.6
	8.07	*npi268a–umc1268*	8.09	*npi438b–dupssr14*	4.9	3.9
TC(M)	3.04	*nc030–phi029*	4.1	*umc1503–bnlg589*	5.6	−3.3
	7.04	*umc1944–umc1295*	8.02	*bnlg1194–umc1304*	10.4	3.8
KM (%)						
TC(B)	2.04	*dupssr27–bnlg381*	2.09	***csu64a–bnlg469b***	10.1	0.6
	7.02	*umc1986–phi034*	9.03	*bnlg1401–phi065*	6.2	−0.5
TC(H)	1.04–1.06	*E35M4910–E35M4902*	7.02	*E35M5507–umc1986*	4.9	0.5
	2.04	*bnlg125–dupssr27*	7.02	*bnlg398–bnlg2203*	6.7	0.5
TC(M)	1.11	*bnlg504–phi064*	7.03	*bnlg339–umc1718*	4.9	−0.5
	2.04	*bnlg125–dupssr27*	9.07	***dupssr29–bnlg619***	4.8	0.4
GY (Mg ha^{-1})						
TC(B)	7.03	*umc2329–umc2331*	10.06	*TC334690–bnl10.13a*	7.9	−0.36
TC(H)	–		–		–	–
TC(M)	1.03	*bnlg439–phi001*	1.04	*bnlg2295–dupssr26*	3.8	0.39
	1.04–1.06	*TC298302–TC344182*	4.08–4.09	*E38M6104–phi093*	5.6	0.41
KW (mg)						
TC(B)	1.08	*bnlg1556–bnlg1025*	5.04	***bnlg557–dupssr10***	11.1	6.6
	2.07	*TC320663–TC333426*	6.01–6.05	*dupssr18–umc1014*	5.6	−7.2
TC(H)	2.04	*bnlg381–phi083*	4.05	*nc005–umc1511*	4.8	−6.5
	2.07	*TC320663–TC333426*	6.01–6.05	*dupssr18–umc1014*	5.0	−5.6
	7.02	*phi034–bnlg398*	9.07	*bnlg1525–bnl5.09a*	3.9	8.6
TC(M)	–		–		–	–
NK (no.)						
TC(B)	5.09	*umc104b–bnlg386*	6.01	***bnlg1371–E38M6103***	7.3	20
TC(H)	2.07	*TC333426–phi127*	3.06	*umc1400–dupssr23*	4.9	−16
TC(M)	–		–		–	–

The intervals corresponding to main-effect QTL or adjacent to them are reported in bold

[a] Estimates of epistatic effects: a positive ss_{ij} value indicates that the parental allelic combinations at interacting loci show mean values higher than recombinant allelic combinations

The significant and positive correlation coefficients observed between TCs for PS, PH, KM, and KW is likely due to their prevailing additive control (Frascaroli et al. 2007), as the relative performances of TCs is expected to change just mildly from one tester to the other. In case of traits with main dominant control (i.e., GY and NK), the relative performances of TC progenies are expected to markedly change from one tester to the other, depending on the testers' genetic constitution, thus explaining the looser correlation between TCs for GY observed in this and in other studies (Melchinger et al. 1998; Austin et al. 2000; Ajmone Marsan et al. 2001). The highest correlation

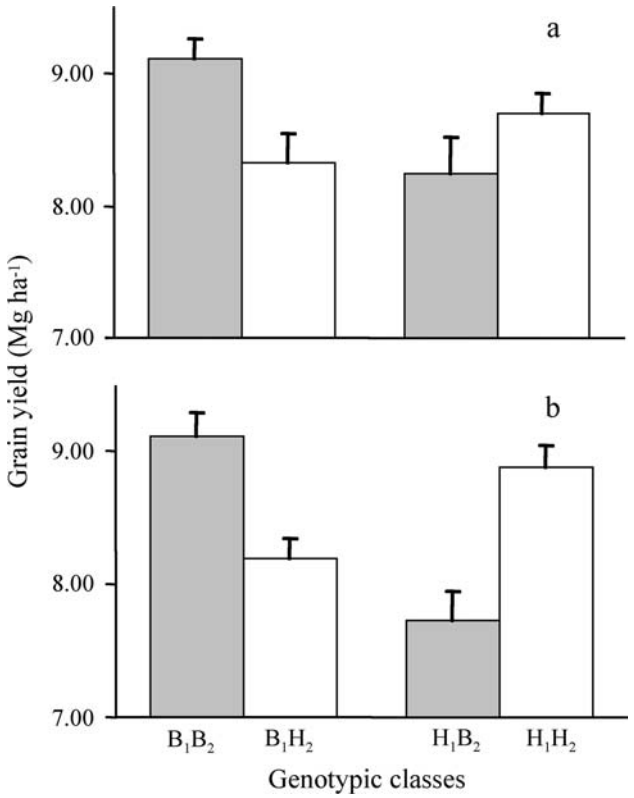

Fig. 2 Mean values for GY in TC(M) of the genotypic classes involving the two QTL (1 and 2) with alleles of B73 (B) and H99 (H). Parental genotypic classes are B_1B_2 and H_1H_2; recombinant genotypic classes are B_1H_2 and H_1B_2. **a** Interaction involving bins 1.03 and 1.04. **b** Interaction involving bins 1.06 and 4.09. *Vertical bars* indicate the standard error of each mean

coefficients found across traits between TC(H) and TC(M) can be explained by considering that H99 and Mo17 belong to the same heterotic group.

Main-effect QTL

Despite the limited size of our mapping population, the number of QTL detected in this study was quite high and comparable to that detected in other TC studies with larger samples (Stuber et al. 1992; Melchinger et al. 1998; Austin et al. 2000; Ajmone Marsan et al. 2001). This finding can be ascribed to the large genetic variation among TCs, as they were produced with RILs obtained from a cross between two inbred lines belonging to different breeding groups. The highest number of QTL was detected in those trait–tester combinations characterized by high h^2 estimates. A positive association between heritability values and number of detected QTL was also observed in other QTL studies (Beavis et al. 1994; Lübberstedt et al. 1997; Melchinger et al. 1998; Austin et al. 2001).

The most effective tester for QTL detection across all traits was the low performing parental inbred H99, whereas the high performing parental inbred B73 was the least effective. However, a distinction should be made between traits with prevailing additive control and traits with prevailing dominance–overdominance control with respect to the relative ability of the three testers to detect QTL. In fact, for the group of traits with prevailing additive genetic control (i.e., PS, PH, KM, and KW), Mo17 was slightly better than H99; in contrast, for the two traits with prevailing dominant genetic control, i.e., GY and NK, H99 revealed a clear superiority, allowing the detection of the higher number of QTL; similar findings were observed for the R^2 of the simultaneous fit of all detected QTL. These findings can be explained by assuming that the high performing parental inbred B73 and the unrelated inbred Mo17 carry, especially for GY and NK, favorable dominant alleles at several loci, thus reducing the ability to detect QTL.

The effects of the detected QTL were more often positive in TC(B) and negative in TC(H) and TC(M); such contrasting findings were particularly evident for GY and NK. Since QTL effects were estimated in TC(B) as the difference between the heterozygote and the homozygote for the B alleles at each locus, the prevalence of positive effects can be explained by assuming that for the detected QTL, the increasing allele in hybrid combination was more often provided by H99, regardless of gene action (additive, dominance, and overdominance); alternatively, it could be assumed that the increasing allele was that of B73, but only in case of overdominance. As to TC(H), the prevalence of negative effects for the detected QTL can be ascribed to increasing alleles provided either by B73, regardless of gene action, or by H99 in case of overdominance. On the whole, these findings thus support the hypothesis that parental inbred B73 does not carry all the increasing alleles in hybrid combination, despite the great superiority of TC(B) over TC(H) for agronomic performance. In TC(M), the prevalence of negative effects can be accounted for by assuming, again, that the increasing alleles in combination with Mo17 alleles were mainly from B73.

Of the 42 QTL detected in only one TC population, 20 were also identified by Frascaroli et al. (2007) who found that 16 of them were characterized by a dominance ratio close to 1. This finding is consistent with the genetic theory, since the dominant alleles carried by the tester are expected to exert a masking effect reducing the genetic variance within a TC population; hence, the QTL for which there is variation in the RIL population can be identified only in the TC population of the tester carrying the recessive alleles.

Of the 28 QTL detected in more than one TC population and showing consistent effects (i.e., of the same sign), 18 were also identified by Frascaroli et al. (2007) and all of them were characterized by a prevalence of additive effects. Again, this finding is consistent with the genetic

theory (for details see Melchinger et al. 1998) because, with prevailing additive gene action, the heterozygote is intermediate between the two homozygotes and, hence, the effect of allele substitution can be revealed in more than one TC population. The frequent overlaps of QTL showing consistency of sign in TC(H) versus TC(M) can account for the positive and highly significant correlations detected between these two TC populations, especially for the four traits with prevailing additive effects. These findings are consistent with the facts that H99 and Mo17 belong to the same heterotic group and that both combine well with BSSS inbred lines such as B73. Also the studies of Beavis et al. (1994), Schön et al. (1994), Lübberstedt et al. (1997), and Melchinger et al. (1998) pointed out that the number of QTL consistently detected with different testers was in accordance with the size of the correlation coefficients among the performances of the TC populations. The QTL with the largest effects were often detected across two or even all three testers. Consistent effects were detected in all three TCs for eight QTL; six of these QTL were also detected by Frascaroli et al. (2007) and all of them were characterized by a prevalence of additive effects, further stressing the importance of such effects to attain QTL consistency across testers. In particular, this was the case of a QTL for GY located in bin 8.05, which was detected, too, in the same RIL population as evaluated per se (Frascaroli et al. 2007). These findings thus imply that selection for this QTL in the material herein investigated can lead to better yield performance both per se and in hybrid combination. This is also in agreement with the study of Austin et al. (2001) who found that QTL with consistent effects across testers are the ones more frequently detected when testing inbred progenies per se.

The four QTL for which the overlap was inconsistent were also identified in the study of Frascaroli et al. (2007) and all of them were characterized by overdominance. Overdominance is a reasonable explanation for the inconsistent effects across testers of these QTL because the allele substitution gives rise to a positive effect in one TC and to a negative effect in the other, the heterozygote being superior to both homozygotes. Our data did not allow the distinction between true- and pseudo-overdominance; however, there is an increasing evidence in the literature showing that much of the heterozygote superiority in maize is mainly due to pseudo-overdominance (Crow 2000).

Epistatic QTL

Some caution should be used as to the interpretation of the epistatic interactions here reported because the size of the investigated population was smaller than that recommended for this type of analysis (Mihaljevic et al. 2005). Both the number and the size of epistatic interactions were smaller than the number and size of the main-effect QTL. These findings thus imply a minor contribution of epistasis to the whole genetic variation, as shown by several studies of classical quantitative genetics (see for a review Hallauer and Miranda 1988). Nevertheless, the presence of even a mild epistasis could have led to some bias in the estimates of QTL effects, especially for those QTL showing inconsistent effects from one tester to the other. The most complex traits such as GY and NK revealed the fewest interactions; a plausible reason for this unexpected finding could be related to their low heritabilities and thus to the low-resolution power in the analyses. For grain yield, very few interactions were detected, too, in the study of Mihaljevic et al. (2005) conducted on four maize populations of lines at different inbreeding levels. According to Wolf and Hallauer (1997), grain yield is affected throughout the plant life cycle by environmental factors which can give rise to various types of environment × epistasis interactions, hence reducing the ability to reveal epistasis across environments.

A substantial balance was observed among testers as to the ability to detect epistatic interactions. Considering TC(M) in more detail, the two interactions found for GY were positive, indicating a superiority of the two parental allelic combinations (especially the ones recalling B73). These findings thus corroborate the hypothesis that the superiority of the check hybrids as compared with TC(M) population is due to epistatic interactions. The presence of allelic combinations fixed in the two parents leading to favorable interactions with Mo17 is not surprising because, at least for the ones involving the B73 and Mo17 genetic backgrounds, positive epistatic effects for GY were already shown (Wolf and Hallauer 1997). One of the two interactions found in TC(M) for GY involved QTL in adjacent bins, i.e., 1.03 and 1.04. Epistatic effects of linked QTL were studied by Cockerham and Zeng (1996) who analyzed materials arising from B73 × Mo17; in particular, they found significant epistasis for grain yield involving regions close to the ones we found, i.e., bins 1.05–1.07. The other interaction detected in the present study in TC(M) for GY concerned bins 1.06 and 4.09; it is noteworthy that an important epistatic interaction for grain yield involving chromosome segments overlapping these two bins was found, too, by Blanc et al. (2006) working with different materials. As to the other traits, the hypothesis that the non-significance between the check hybrids' mean and TC(M) mean was due to epistatic effects of opposite sign was validated at least for PH and KM.

Conclusion

In conclusion, the three testers proved to markedly influence the QTL detection and the estimate of their effects in

the RILs population herein examined; in addition, such an influence varied depending on the investigated trait. For traits with mainly additive control, the unrelated inbred Mo17 was slightly more effective than the poor performing parental inbred H99 and both exceeded the high performing parental inbred B73. In contrast, for traits characterized by prevailing dominance–overdominance gene action, H99 was notably the most effective, whereas Mo17 was the least effective. The three testers allowed the detection of a few epistatic interactions, rarely involving QTL with main effects.

Several QTL were detected in two or three TC populations, showing consistent effects, especially in case of QTL with prevailing additive gene action; the reliability of their estimates across testers suggests that these QTL can be suitable for the application of marker assisted selection (MAS). Only in few instances the QTL effects were inconsistent; for these QTL, MAS should be conducted by referring to a well-defined and specific tester, so as to fully exploit the potentiality of the allelic and non-allelic interactions with the tester's genetic background.

Acknowledgments The study was conducted with the financial support of the Italian Ministry of University and Research, PRIN (*Progetti di Ricerca di Interesse Nazionale*) Project: "Modern approaches of quantitative genetics for the analysis of agronomic traits in maize".

References

Ajmone Marsan P, Gorni C, Chittò A, Redaelli R, van Vijk R, Stam P, Motto M (2001) Identification of QTLs for grain yield and grain-related traits of maize (*Zea mays* L.) using an AFLP map, different testers, and cofactor analysis. Theor Appl Genet 102:230–243

Austin DF, Lee M, Veldboom LR, Hallauer AR (2000) Genetic mapping in maize with hybrid progeny across testers and generations: grain yield and grain moisture. Crop Sci 40:30–39

Austin DF, Lee M, Veldboom LR (2001) Genetic mapping in maize with hybrid progeny across testers and generations: plant height and flowering. Theor Appl Genet 102:163–176

Beavis WD, Smith OS, Grant D, Fincher R (1994) Identification of quantitative trait loci using a small sample of topcrossed and F4 progeny from maize. Crop Sci 34:882–896

Blanc G, Charcosset A, Mangin B, Gallais A, Moreau L (2006) Connected populations for detecting quantitative trait loci and testing for epistasis: an application in maize. Theor Appl Genet 113:206–224

Burr B, Burr FA (1991) Recombinant inbreds for molecular mapping in maize: theoretical and practical considerations. Trends Genet 7:55–60

Churchill GA, Doerge RW (1994) Empirical threshold values for quantitative trait mapping. Genetics 138:963–971

Cockerham CC, Zeng ZB (1996) Design III with marker loci. Genetics 143:1437–1456

Crow JF (2000) The rise and fall of overdominance. Plant Breed Rev 17:225–257

Frascaroli E, Canè MA, Landi P, Pea G, Gianfranceschi L, Villa M, Morgante M, Pè ME (2007) Classical genetic and quantitative trait loci analyses of heterosis in a maize hybrid between two elite inbred lines. Genetics 176:625–644

Frova C, Krajewski P, Di Fonzo N, Villa M, Sari-Gorla M (1999) Genetic analysis of drought tolerance in maize by molecular markers. I. Yield components. Theor Appl Genet 99:280–288

Groh S, González-de-León D, Khairallah MM, Jiang C, Bergvinson D, Bohn M, Hoisington DA, Melchinger AE (1998) QTL mapping in tropical maize: III. Genomic regions for resistance to *Diatraea* spp. and associated traits in two RIL populations. Crop Sci 38:1062–1072

Hallauer AR (1990) Methods used in developing maize inbreds. Maydica 35:1–16

Hallauer AR, Miranda JB (1988) Quantitative genetics in maize breeding. Iowa State University Press, Ames

Knapp SJ, Stroup WW, Ross WM (1985) Exact confidence intervals for heritability on a progeny mean basis. Crop Sci 25:192–194

Lande R, Thompson R (1990) Efficiency of marker-assisted selection in the improvement of quantitative traits. Genetics 124:743–756

Li ZK, Luo LJ, Mei HW, Wang DL, Shu QY, Tabien R, Zhong DB, Ying CS, Stansel JW, Khush GS, Paterson AH (2001) Overdominant epistatic loci are the primary genetic basis of inbreeding depression and heterosis in rice. I. Biomass and grain yield. Genetics 158:1737–1753

Livini C, Ajmone-Marsan P, Messmer MM, Melchinger AE, Motto M (1992) Genetic diversity of maize inbred lines within and among heterotic groups revealed by RFLP. Theor Appl Genet 84:17–25

Lu H, Bernardo R (2001) Molecular marker diversity among current and historical maize inbreds. Theor Appl Genet 103:613–617

Lübberstedt T, Melchinger AE, Klein D, Degenhardt H, Paul C (1997) QTL mapping in test crosses of European flint lines of maize 2. Comparisons of different testers for forage quality traits. Crop Sci 37:1913–1922

Melchinger AE, Utz HF, Schön CC (1998) Quantitative trait locus (QTL) mapping using different testers and independent population samples in maize reveals low power of QTL detection and large bias in estimates of QTL effects. Genetics 149:383–403

Mihaljevic R, Utz HF, Melchinger AE (2005) No evidence for epistasis in hybrid and per se performance of elite european flint maize inbreds from generation means and QTL analyses. Crop Sci 45:2605–2613

SAS INSTITUTE (1996) SAS users guide: statistic. SAS Institute, Cary

Schön CC, Melchinger AE, Boppenmaier J, Brunklaus-Jung E, Herrmann RG, Seitzer JF (1994) RFLP mapping in maize: quantitative trait loci affecting testcross performances of elite european flint lines. Crop Sci 34:378–389

Stuber CW, Lincoln SE, Wolff DW, Helentjaris T, Lander ES (1992) Identification of genetic factors contributing to heterosis in a hybrid from two elite maize inbred lines using molecular markers. Genetics 132:823–839

Utz HF, Melchinger AE (1996) PLABQTL: a program for composite interval mapping of QTL. J Quant Trait Loci 2:1–5

Wang DL, Zhu J, Li ZK, Paterson AH (1999) Mapping QTL with epistatic effects and QTL × environment interactions by mixed model approaches. Theor Appl Genet 99:1255–1264

Wolf DP, Hallauer AR (1997) Triple testcross analysis to detect epistasis in maize. Crop Sci 37:763–770

Zeng ZB (1994) Precision mapping of quantitative trait loci. Genetics 136:1457–1468