Theor Appl Genet (2013) 126:773–789
DOI 10.1007/s00122-012-2017-1

ORIGINAL PAPER

Correlations and comparisons of quantitative trait loci with family per se and testcross performance for grain yield and related traits in maize

Bo Peng · Yongxiang Li · Yang Wang · Cheng Liu · Zhizhai Liu · Yan Zhang · Weiwei Tan · Di Wang · Yunsu Shi · Baocheng Sun · Yanchun Song · Tianyu Wang · Yu Li

Received: 26 April 2012 / Accepted: 5 November 2012 / Published online: 27 November 2012

Abstract Simultaneous improvement in grain yield and related traits in maize hybrids and their parents (inbred lines) requires a better knowledge of genotypic correlations between family per se performance (FP) and testcross performance (TP). Thus, to understand the genetic basis of yield-related traits in both inbred lines and their testcrosses, two $F_{2:3}$ populations (including 230 and 235 families, respectively) were evaluated for both FP and TP of eight yield-related traits in three diverse environments. Genotypic correlations between FP and TP, $\hat{r}_{\mathrm{g}}$ (FP, TP), were low (0–0.16) for grain yield per plant (GYPP) and kernel number per plant (KNPP) in the two populations, but relatively higher (0.32–0.69) for the other six traits with additive effects as the primary gene action. Similar results were demonstrated by the genotypic correlations between observed and predicted TP values based on quantitative trait loci positions and effects for FP, $\hat{r}_{\mathrm{g}}$ (M_{FP}, Y_{TP}). A total of 88 and 35 QTL were detected with FP and TP, respectively, across all eight traits in the two populations. However, the genotypic variances explained by the QTL detected in the cross-validation analysis were much lower than those in the whole data set for all traits. Several common QTL between FP and TP that accounted for large phenotypic variances were clustered in four genomic regions (bin 1.10, 4.05–4.06, 9.02, and 10.04), which are promising candidate loci for further map-based cloning and improvement in grain yield in maize. Compared with publicly available QTL data, these QTL were also detected in a wide range of genetic backgrounds and environments in maize. These results imply that effective selection based on FP to improve TP could be achieved for traits with prevailing additive effects.

Introduction

Single-cross hybrids have contributed greatly to improvement in grain yield since the 1930s in the United States (Duvick 1999) and since the 1960s in China (Ci et al. 2011; Li et al. 2011; Wang et al. 2011). One approach to develop superior hybrids is the parallel evaluation of both inbred lines per se and testcrosses produced by crossing these lines with proper testers (Bekavac et al. 2008). An elite inbred line should have both high grain yield and good combining ability (Hallauer and Miranda 1988; Bekavac et al. 2008). Thus, the simultaneous improvement of family per se performance and its combining ability is critical for maize breeding.

An indirect improvement in testcross performance (TP) in early generations by selecting for line per se performance (FP) is desirable because testcrossing and phenotypic

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B. Peng, Y. Li and Y. Wang had equal contribution.

Communicated by M. Frisch.

B. Peng · Y. Li · Y. Wang · Z. Liu · Y. Zhang · W. Tan · D. Wang · Y. Shi · Y. Song · T. Wang (✉) · Y. Li (✉)
Institute of Crop Science, Chinese Academy of Agricultural Sciences, Beijing 100081, China
e-mail: wangtianyu@263.net

Y. Li
e-mail: yuli@mail.caas.net.cn

B. Peng
Tianjin Crops Research Institute, Tianjin 300112, China

C. Liu · B. Sun
Institute of Food Crops, Xinjiang Academy of Agricultural Sciences, Urumqi 830000, China

Z. Liu
Southwest University, Chongqing 400716, China

evaluation of TP are often expensive and time-consuming (Mihaljevic et al. 2005). The genotypic correlation between FP and TP, $\hat{r}_g$ (FP, TP), is crucial for determining selection efficiency for the indirect improvement of TP based on FP. Several factors, such as recessive genes with detrimental effects in homozygotes, the level of heterozygosity of FP, overdominance, epistasis, and linkage, may decrease the estimates of $\hat{r}_g$ (FP, TP) (Smith 1986). $\hat{r}_g$ (FP, TP) for grain yield, grain moisture, kernel weight, protein concentration in grain, plant height, stay green, anthesis-silking interval, and stalk water content were investigated in previous studies (Austin et al. 2000; Mihaljevic et al. 2005; Bekavac et al. 2008). However, knowledge regarding $\hat{r}_g$ (FP, TP) for kernel traits to-date is sparse.

In the past two decades, molecular-marker techniques have greatly facilitated the investigation of quantitatively inherited complex traits (Bernardo 2008). More than 2200 QTL conferring a wide range of traits in maize have been registered in the Maize Genetics and Genomics Database (http://www.maizegdb.org/). However, most of those QTL were mapped based on FP, such as those of recombinant inbred lines, double-haploid lines, or $F_{2:3}$ populations. Information about QTL detected with TP and data on whether expression of QTL detected with FP are transmissible to their testcrosses have been particularly rare (Mihaljevic et al. 2004, 2005; You et al. 2006). Whether MAS on TP based on QTL detected with FP is feasible and efficient remains unclear.

One primary objective of QTL studies is to identify marker-QTL associations for MAS in the improvement of quantitative traits. In addition to several factors such as different QTL segregations in different mapping populations, QTL × environment interactions, QTL × genetic background interactions, and the overestimated proportion of genetic variance explained by QTL also restricted the application of MAS (Schön et al. 2004; Francia et al. 2005; Bernardo 2008; Buckler et al. 2009). Limited sample sizes of mapping populations have led to fewer detected QTL and an upward bias in the estimated effects of the few QTL detected (Utz et al. 2000; Francia et al. 2005; Bernardo 2008). To overcome the inflated proportion of genotypic variance explained by QTL due to genotypic and environmental sampling, the cross-validation (CV) method can be used to obtain more realistic estimations (Melchinger et al. 1998; Utz et al. 2000).

In this study, two $F_{2:3}$ populations derived from two crosses of foundation inbred lines of maize were evaluated for both FP and TP of grain yield and seven related traits in three environments. The objectives of this study were to (1) estimate the phenotypic and genotypic correlations between FP and TP for all eight traits, (2) identify and compare QTL with FP and TP for grain yield and related traits, and (3) assess the bias of the proportion of genotypic variance explained by QTL using CV.

Materials and methods

Plant materials

Qi319, Ye478, Huangzaosi, and Mo17 are foundation inbred lines in China maize breeding and represent the P, Reid, Tangsipingtou, and Lancaster heterotic groups, respectively (Wang et al. 2008). A great number of commercial single-cross hybrids were developed by crossing two of these four lines or their derived descendents in China (Ci et al. 2011; Li et al. 2011; Wang et al. 2011). In 2006, random F_2 plants derived from the two crosses, Qi319 × Huangzaosi (hereafter Q/H) and Ye478 × Huangzaosi (Y/H), were self-fertilized to generate 230 $F_{2:3}$ families for Q/H (hereafter Q/H-FP) and 235 $F_{2:3}$ families for Y/H (Y/H-FP), respectively. In 2007, testcross seeds were produced by controlled hand pollinations by crossing 15 plants of each family of the two populations as female parents with the inbred tester “Mo17.” A total of 230 and 235 testcrosses for Q/H (Q/H-TP) and Y/H (Y/H-TP), respectively, were obtained. Simultaneously, the $F_{2:3}$ families were multiplied by full-sib mating for the next growing season. The parents of the two populations (i.e., Qi319, Ye478, and Huangzaosi) were also crossed to Mo17.

Field experiments

The FP and TP of the two populations and their parents were evaluated in 2008 at three locations: Xinxiang of Henan province (35.19°N, 113.53°E) in central China, Beijing (39.48°N, 116.28°E) in northern China, and Urumqi of Xinjiang province (43.47°N, 87.39°E) in western China. These locations, which have diverse agro-ecological conditions, represented three main maize growing regions in China. Each location was considered to be an environment. The entries were summer sown in Henan and spring sown in Beijing and Xinjiang in randomized complete blocks with two replicates. Each plot comprised a single row 4 m long, spaced 0.6 m apart, and with a density of 52, 500 plants per ha. The trials were managed following local standard practices.

Phenotypic data were collected for the following traits: (1) grain yield per plant (GYPP, g), the average of five plants in the middle of each row; (2) 100-kernel weight (KWEI, g), the average of three measurements of the weight of 100 randomly selected kernels; (3) kernel number per plant (KNPP), calculated by the formula KNPP = 100 × GYPP/KWEI; (4) 100-kernel volume

(KVOL, ml), the average of three measurements of the volume of 100 randomly selected kernels; (5) kernel density (KDEN, g/ml), calculated by dividing the 100-kernel weight by the 100-kernel volume; (6) 10-kernel length (KLEN, cm), the average of five measurements of the length of 10 kernels in the middle of an ear; (7) 10-kernel width (KWID, cm), the average of five measurements of the width of 10 kernels in the middle of an ear; and (8) 10-kernel thickness (KTHI, cm), the average of five measurements of the thickness of 10 kernels in the middle of an ear.

Statistical analysis of phenotypic data

Analysis of variance was conducted by PROC ANOVA in PLABSTAT (Utz 1997) with genotype, environment, replication within environment, and interaction between environment and genotype as random effects in the statistical model. Variance components σ_g^2 (genotypic variance), σ_{ge}^2 (genotype-by-environment interaction variance), and σ_ε^2 (experimental error) and their standard error (SE) were calculated. The broad-sense heritability (h^2) for each trait was calculated on a plot basis as $h^2 = \sigma_g^2/(\sigma_g^2 + \sigma_{ge}^2/e + \sigma_\varepsilon^2/re)$, where e and r are the numbers of environments and replicates within each environment, respectively (Hallauer and Miranda 1988). The 95 % confidence intervals (CI) of h^2 were also calculated. The phenotypic correlations, $\hat{r}_p$ (FP, TP), and genotypic correlations, $\hat{r}_g$ (FP, TP), between FP and TP for a given trait were evaluated with the PLABSTAT software (Utz 1997).

Genetic map construction

Genomic DNA was extracted from young leaves of the $F_{2:3}$ families (15 plants per $F_{2:3}$ family as a bulk) and their parents using the CTAB method (Chen and Ronald 1999). More than 500 primer pairs from http://www.maizegdb.org/ were used to survey SSR polymorphisms between the parents for each population. Next, a total of 194 (for Q/H) and 159 (for Y/H) polymorphic SSR primer pairs were applied to construct two genetic maps using the Mapmaker software, version 3.0 (Lander et al. 1987). The recombination frequencies were converted to genetic distance using the Haldane mapping function. The maps covered all ten maize chromosomes with total genome sizes of 2,493.7 cM (Q/H) and 3,168.9 cM (Y/H). The average marker intervals were 12.9 and 20.1 cM for Q/H and Y/H, respectively.

QTL analysis

QTL mapping and estimation of their effects were performed using composite interval mapping and means across environments with PLABQTL (Utz and Melchinger 1996). The additive-dominance and additive genetic models were used for the analysis of FP and TP, respectively, as described by Utz et al. (2000) and Mihaljevic et al. (2005). Cofactors were selected by stepwise regression following PLABQTL's default. An LOD score of 2.5 was selected as the threshold to declare the presence of a significant QTL. Estimates of QTL positions were obtained at the position at which the LOD score assumed its maximum. The proportion of the phenotypic variance explained by all detected QTL was determined by the adjusted coefficient of determination of regression (R^2_{adj}) fitting a model including all detected QTL (Utz et al. 2000). The proportion of the genotypic variance explained by all detected QTL for a given trait was estimated as $p = R^2_{adj}/h^2$, where h^2 is the heritability of the trait.

CV

To test the effect of genotypic sampling, fivefold CV (CV/G) as described by Utz et al. (2000) was performed to estimate, without bias, the proportion of genotypic variance explained by all QTL (p) for a given trait. The whole data set containing the entry means across environments for each population was randomly split into $k = 5$ genotypic subsets. Four subsets were combined to form the estimation set (ES) for QTL detection and the estimation of genetic effects. The remaining subset formed the test set (TS) to validate the predictions derived from the ES and calculate $p_{TS.ES}$ from the adjusted squared correlation coefficient between the phenotypic entry means observed in TS and the predicted genotypic values on the basis of QTL estimate in the ES, divided by the heritability of the trait under investigation. A total of 1,000 replicated CV/G runs were performed with 200 randomizations to assign genotypes to the respective subsets. The $p_{TS.ES}$ was obtained from the median across the 1,000 runs. For QTL in bin 4.05–4.06 and its CI estimated using the whole data set, the frequency of QTL detection across the CV/G runs was calculated by counting the number of CV/G runs in which a QTL was located within that CI The frequency of QTL detection gave an estimation of the precision of QTL localization (Utz et al. 2000).

Congruency of QTL for FP and TP

The congruency of QTL with FP and TP of a given trait in the same population was assessed by two approaches (Mihaljevic et al. 2005): (1) counting the number of common QTL; QTL were considered common if they were located within a 20-cM distance, irrespective of the sign of estimated QTL effects (Melchinger et al. 1998); and (2) the genotypic correlation between predicted and observed TP,

$\hat{r}_g$ (M_{FP}, Y_{TP}), where M_{FP} is the predicted value of a family based on the QTL positions and effects estimated from QTL with FP in a given population, and Y_{TP} is the observed TP of this family (Utz et al. 2000).

QTL for a particular trait that were found in the same or adjacent bins across the two populations (Q/H and Y/H) were declared common across populations (Schön et al. 2010).

Results

FP and TP

Significant differences ($P < 0.05$) among the FP of the three parental lines in terms of GYPP, KNPP, KWEI, KVOL, KLEN, and KTHI were detected. There was no significant difference between Huangzaosi and Qi319/Ye478 for GYPP ($P < 0.05$), but the methods by which a high yield was achieved differed among the three parental lines. Huangzaosi had a higher KNPP than did the other two parents, while Qi319 and Ye478 had higher KWEI, KVOL, KLEN, and KTHI (Fig. 1). Four of the eight traits (i.e., GYPP, KNPP, KLEN, and KTHI) differed significantly ($P < 0.05$) among the TP of the three parental lines (data not shown).

The mean values of TP for GYPP, KNPP, KWEI, KDEN, KVOL, KLEN, KWID, and KTHI were 72.1, 47.9, 15.2, 1.4, 13.6, 16.5, 3.6, and −9.2 % higher than the corresponding values of the FP across the two populations. The phenotypic values of both types of progeny (i.e., $F_{2:3}$ families and their testcrosses) for all traits exhibited broad variation and an approximately normal distribution in each population, indicating both that all traits had polygenic characteristics and the feasibility of QTL mapping (Fig. 1). KNPP, KDEN, KWID, and KTHI exhibited wider distributions in FP than in TP, while GYPP exhibited wider distributions in TP than in FP (Fig. 1). However, there was no consistency in the distribution of FP and TP for KWEI, KVOL, and KLEN between the two populations.

Genetic variation between both types of progenies was significant ($P < 0.01$) for all traits in each population and significant ($P < 0.01$) σ^2_{ge} for all traits, with the exception of KNPP, for TP in Y/H, KDEN for TP in Q/H, FP and TP in Y/H, KWID for FP and TP in Y/H, and KTHI for FP in Y/H (Table 1). The genetic variance for FP was higher than that for TP for all traits in both populations, with the exceptions of GYPP and KNPP in the two populations and KLEN in Q/H. Heritabilities calculated across environments ranged from 46.33 % (for FP of KDEN in Y/H) to 84.69 % (for FP of KVOL in Y/H). In contrast to those of GYPP and KNPP, the heritabilities of the other traits, excluding that of KDEN, were relatively high (>0.6). The confidence interval of heritability for all traits was 6.9–24.2 % in the two populations.

Correlations between FP and TP for grain yield and related traits

For both types of progeny, significant and positive phenotypic and genotypic correlations were observed between GYPP and KNPP, KWEI, KVOL, KLEN, and KWID in the two populations (Table 2), suggesting that GYPP and the above-mentioned traits were to some extent regulated by a common genetic mechanism. The genotypic correlation between GYPP and KDEN for FP was in a different direction from that for TP in the two populations. The genotypic correlations between GYPP and KTHI for FP and TP were also in different directions in Q/H, but the influence of KTHI on GYPP was negligible in Y/H.

The phenotypic correlation coefficient between FP and TP, $\hat{r}_p$ (FP, TP), was low and not significant ($P > 0.05$) for GYPP and KNPP, but intermediate (0.26–0.48) and significant ($P < 0.01$) for KWEI, KDEN, KVOL, KLEN, KWID, and KTHI (Table 3). The genotypic correlation coefficients between FP and TP, $\hat{r}_g$ (FP, TP), were significant ($P < 0.05$) for all traits with the exception of KNPP. Similar to $\hat{r}_p$ (FP, TP), $\hat{r}_g$ (FP, TP) was low for GYPP and KNPP (0–0.16) and intermediate for the other six traits (0.32–0.69). $\hat{r}_g$ (FP, TP) was generally higher than $\hat{r}_p$ (FP, TP) for all traits in the two populations.

QTL analysis based on FP and TP

A total of 123 of QTL affecting the eight traits with FP and TP in the two populations were identified (Tables 3, 4, 5; Fig. 2), among which 52, 23, 36, and 12 QTL were detected in Q/H-FP, Q/H-TP, Y/H-FP, and Y/H-TP, respectively, which explained the 18.3–52.8 % (31.7–66.4 %), 10.4–23.4 % (15.7–33.3 %), 3.2–38.4 % (6.5–52 %), and 3.7–20 % (5.6–29.9 %) phenotypic (genotypic) variances, respectively (Tables 4, 5). CV analysis resulted in a substantial reduction in $p_{TS.ES}$ values compared with p_{DS} values because the genotypic variances explained by all detected QTL were 5.8–43.5, 4.1–13.1, 0–27, and 0–22 % in Q/H-FP, Q/H-TP, Y/H-FP, and Y/H-TP, respectively.

The types of gene action controlling the eight traits were also revealed by QTL mapping (Tables 4, 5). More than 60 % of the QTL for GYPP and KNPP were controlled by dominant effects for FP, whereas most of the other traits were affected by additive QTL in the two populations.

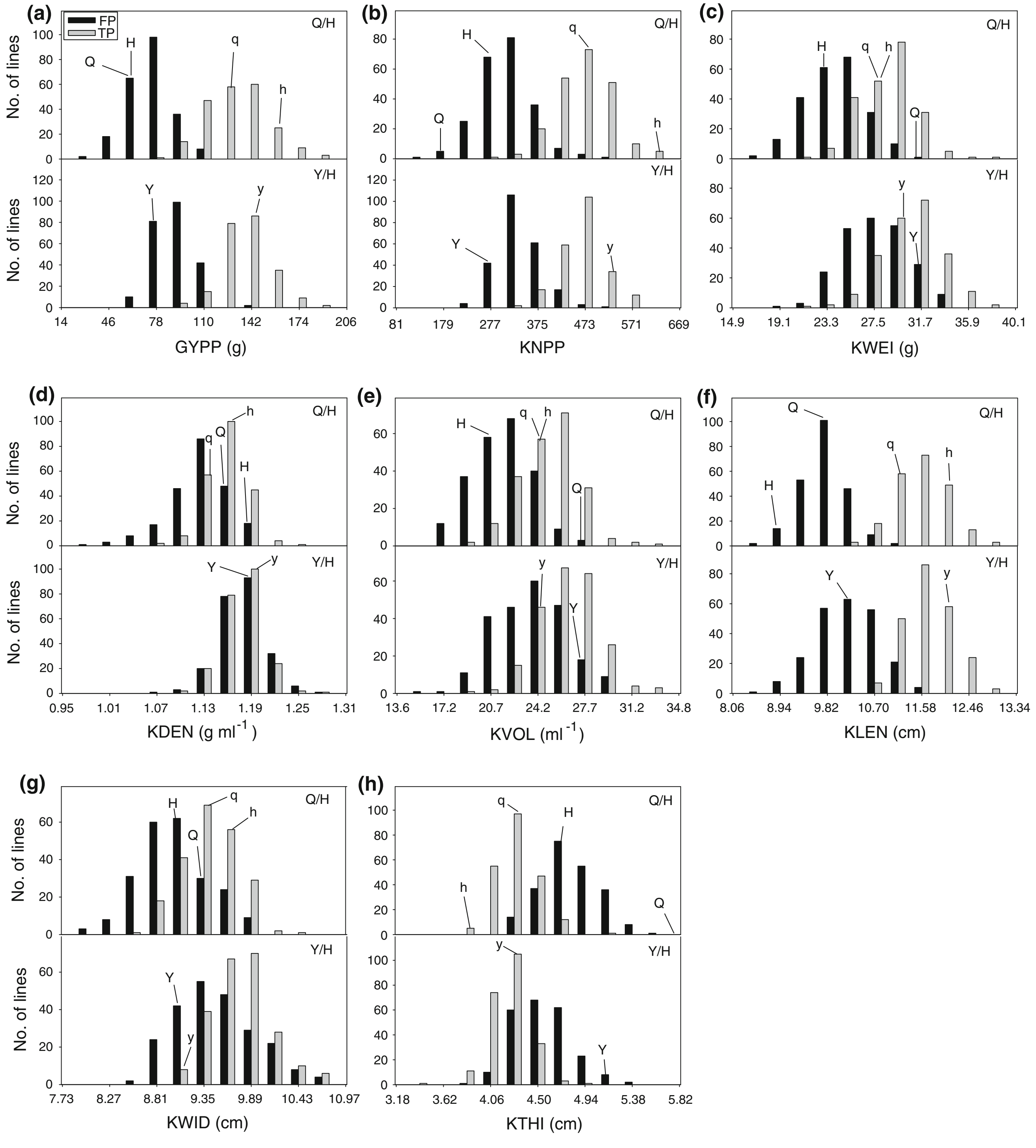

Fig. 1 Phenotypic distribution for eight traits evaluated for FP and TP of the two populations across three environments. **a** Grain yield per plant (GYPP), **b** kernel number per plant (KNPP), **c** 100-kernel weight (KWEI), **d** kernel density (KDEN), **e** 100-kernel volume (KVOL), **f** 10-kernel length (KLEN), **g** 10-kernel width (KWID), and **h** 10-kernel thickness (KTHI). Means for FP (*uppercase*) and TP (*lowercase*) of Qi319 (*Q*, *q*), Ye478 (*Y*, *y*), and Huangzaosi (*H*, *h*) are labeled

Comparison of QTL across two kinds of progenies and the two populations

For the eight traits, ten and six common QTL (referring to QTL located within a 20-cM distance) between FP and TP were found in Q/H and Y/H, respectively (Tables 3, 4, 5; Fig. 2). The number of common QTL between FP and TP ranged from 0 to 2 for each of the eight traits in the two populations. However, KNPP, KDEN, and KTHI were not found to be common QTL in Y/H.

Table 1 Variance components and heritability for family per se performance (FP) and their testcross performance (TP) of the two populations with the tester Mo17 evaluated for grain yield and related traits of maize in three environments

Trait	Progeny type	Population	Mean	σ^2_{g}	σ^2_{ge}	σ^2_{ε}	h^2 (%)	95 % CI on h^2
GYPP	FP	Q/H	66.03	119.3 ± 20.2**	141.8 ± 18.6**	240.5 ± 13.3	57.73	46.77, 66.15
		Y/H	82.52	91.8 ± 16.1**	52.4 ± 17.3**	340.8 ± 18.7	55.28	43.88, 64.07
	TP	Q/H	123.25	270.4 ± 40.9**	181.5 ± 32.4**	499.6 ± 28.0	65.29	56.05, 72.34
		Y/H	129.94	128.3 ± 25.8**	77.4 ± 31.6**	634.0 ± 35.6	49.39	36.28, 59.45
KNPP	FP	Q/H	285.46	1,852.9 ± 305.5**	1,879.1 ± 277.2**	3,921.5 ± 216.9	59.14	48.54, 67.28
		Y/H	313.75	969.7 ± 193.2**	664.5 ± 234.8**	4,673.5 ± 257.3	49.22	36.27, 59.20
	TP	Q/H	444.26	2,290.2 ± 341.4**	1,030.8 ± 277.1**	4,988.8 ± 279.3	66.09	57.06, 72.98
		Y/H	440.09	1,214.6 ± 213.7**	367.3 ± 239.5	5,064.2 ± 285.1	55.69	44.21, 64.50
KWEI	FP	Q/H	23.15	5.63 ± 0.67**	1.85 ± 0.32**	5.01 ± 0.27	79.51	74.19, 83.59
		Y/H	26.64	6.72 ± 0.76**	1.57 ± 0.31**	5.37 ± 0.29	82.59	78.15, 86.01
	TP	Q/H	27.53	3.86 ± 0.61**	3.07 ± 0.52**	7.74 ± 0.43	62.52	52.54, 70.13
		Y/H	29.68	4.56 ± 0.63**	1.97 ± 0.46**	8.28 ± 0.45	69.13	61.15, 75.27
KDEN	FP	Q/H	1.11	0.0011 ± 0.0001**	0.0003 ± 0.0001**	0.0015 ± 0.0001	75.99	69.76, 80.77
		Y/H	1.16	0.0004 ± 0.0001**	0.0001 ± 0.0001	0.0025 ± 0.0001	46.33	32.64, 56.87
	TP	Q/H	1.14	0.0004 ± 0.0001**	0.0001 ± 0.0001	0.0015 ± 0.0001	59.90	49.22, 68.04
		Y/H	1.16	0.0003 ± 0.0001**	0.0001 ± 0.0001	0.0017 ± 0.0001	48.19	34.79, 58.49
KVOL	FP	Q/H	20.81	4.12 ± 0.49**	1.14 ± 0.21**	3.40 ± 0.19	81.31	76.47, 85.04
		Y/H	22.88	5.47 ± 0.60**	1.06 ± 0.22**	3.81 ± 0.21	84.69	80.79, 87.70
	TP	Q/H	24.07	3.20 ± 0.46**	1.90 ± 0.33**	5.05 ± 0.28	68.43	60.03, 74.84
		Y/H	25.51	3.31 ± 0.46**	1.47 ± 0.33**	5.84 ± 0.32	69.32	61.39, 75.42
KLEN	FP	Q/H	9.55	0.139 ± 0.017**	0.038 ± 0.010**	0.185 ± 0.010	76.08	69.88, 80.85
		Y/H	10.00	0.268 ± 0.031**	0.029 ± 0.015*	0.321 ± 0.017	80.93	76.07, 84.68
	TP	Q/H	11.31	0.180 ± 0.023**	0.049 ± 0.012**	0.211 ± 0.012	77.78	71.86, 82.29
		Y/H	11.46	0.123 ± 0.018**	0.039 ± 0.015**	0.288 ± 0.016	66.82	58.23, 73.42
KWID	FP	Q/H	8.88	0.129 ± 0.015**	0.023 ± 0.007**	0.128 ± 0.007	81.64	76.87, 85.30
		Y/H	9.36	0.163 ± 0.019**	0.014 ± 0.011	0.229 ± 0.012	79.12	73.79, 83.22
	TP	Q/H	9.26	0.074 ± 0.010**	0.017 ± 0.008*	0.153 ± 0.009	70.34	62.44, 76.36
		Y/H	9.64	0.076 ± 0.011**	0.005 ± 0.005	0.217 ± 0.012	66.85	58.27, 73.44
KTHI	FP	Q/H	4.70	0.058 ± 0.007**	0.011 ± 0.004**	0.086 ± 0.005	76.20	70.02, 80.94
		Y/H	4.44	0.052 ± 0.007**	0.003 ± 0.005	0.102 ± 0.006	74.06	67.45, 79.16
	TP	Q/H	4.18	0.027 ± 0.004**	0.009 ± 0.003**	0.045 ± 0.003	72.06	64.62, 77.73
		Y/H	4.11	0.022 ± 0.003**	0.004 ± 0.003*	0.056 ± 0.003	66.60	57.95, 73.24

*, ** Significance at $P < 0.05$ and 0.01, respectively

Table 2 Phenotypic and genotypic correlations between seven agronomic traits and grain yield across three environments

Population	Type of progeny	KNPP	KWEI	KDEN	KVOL	KLEN	KWID	KTHI
Q/H	FP	0.85** (0.79**)	0.46** (0.51**)	0.35** (0.40**)	0.39** (0.42**)	0.62** (0.68**)	0.31** (0.36**)	−0.23** (−0.29**)
	TP	0.83** (0.85**)	0.57** (0.58**)	−0.07 (−0.24**)	0.59** (0.61**)	0.65** (0.67**)	0.32** (0.33**)	0.21** (0.22**)
Y/H	FP	0.73** (0.60**)	0.46** (0.57**)	−0.04 (−0.20*)	0.46** (0.59**)	0.60** (0.71**)	0.35** (0.42**)	−0.01 (0.08)
	TP	0.70** (0.59**)	0.54** (0.53**)	0.16* (0.23*)	0.51** (0.50**)	0.52** (0.59**)	0.28** (0.31**)	−0.03 (−0.08)

*, ** Significance at $P < 0.05$ and 0.01, respectively. Phenotypic or genotypic correlation coefficients were showed out or within bracket, respectively

For all eight traits, only 5 of 20 and 4 of 9 major QTL ($R^2 > 10$ %) with FP were also detected with TP in Q/H and Y/H, respectively. Many QTL accounting for a large phenotypic variation were detected with FP, but not with TP in the same population, such as QTL for KWID and KVOL in bin 10.07, and QTL for KWEI in bin 2.06 in Q/H, and QTL for KTHI in bin 1.09, and QTL for KVOL in bin 9.03 in Y/H. Six of 10 and 2 of 3 QTL with larger

Table 3 Phenotypic ($\hat{r}_p$) and genotypic ($\hat{r}_g$) correlations between family per se performance (FP) and testcross performance (TP), the number of quantitative trait loci (QTL) detected with FP and TP as well as the number of common QTL for grain yield and yield-related traits of maize, and the genotypic correlation between FP and TP based on estimated QTL, $\hat{r}_g$ (M_{FP}, Y_{TP})

Population	$\hat{r}_p$ (FP, TP)	$\hat{r}_g$ (FP, TP)	$\hat{r}_g$ (M_{FP}, Y_{TP})[a]	No. of QTL (FP)	No. of QTL (TP)	No. of common QTL
	GYPP					
Q/H	0.09	0.16*	0	3	1	1
Y/H	0.11	0.15*	0.47**	4	2	1
	KNPP					
Q/H	0.08	0.11	0.45**	4	2	1
Y/H	0	0	0	1	0	0
	KWEI					
Q/H	0.39**	0.50**	0.67**	11	3	1
Y/H	0.44**	0.57**	0.52**	7	2	1
	KDEN					
Q/H	0.41**	0.57**	0.77**	8	4	2
Y/H	0.32**	0.69**	0.44**	5	1	0
	KVOL					
Q/H	0.41**	0.50**	0.60**	10	3	1
Y/H	0.48**	0.62**	0.48**	5	2	1
	KLEN					
Q/H	0.26**	0.32**	0.33**	4	3	1
Y/H	0.48**	0.66**	0.58**	6	2	2
	KWID					
Q/H	0.42**	0.50**	0.53**	5	4	1
Y/H	0.39**	0.54**	0.42**	5	2	1
	KTHI					
Q/H	0.48**	0.60**	0.47**	7	3	2
Y/H	0.39**	0.52**	0.27**	3	1	0

[a] Correlation between the observed TP and predicted genotypic values on the basis of QTL positions and effects derived from FP, divided by the heritability

*, ** Significance at $P < 0.05$ and 0.01, respectively

effects with TP ($R^2 > 8.0$ %) across the eight traits were not detected with FP in Q/H and Y/H, respectively.

Some QTL common to both FP and TP explained the larger phenotypic variance in both types of progeny. They included the QTL for KDEN (explaining 22.6 and 5.9 % phenotypic variance with FP and TP, respectively) and KTHI (9.4 and 8.1 %) in bin 1.10, the QTL for KLEN in bin 4.03 (12.7 and 7.1 %), the QTL for KDEN in bin 4.06 (10.9 and 9.1 %), and the QTL for GYPP (7.9 and 13.0 %) and KTHI (8.5 and 10.9 %) in bin 10.04 in Q/H; and the QTL for KLEN (17.2 and 5.3 %), KVOL (11.9 and 7.3 %), and KWEI (11.4 and 7.9 %) in bin 4.05 and the QTL for KLEN (11.1 and 16.9 %) in bin 9.02 in Y/H. For these common QTL, the favorable alleles were conferred by the same parents. However, the magnitudes of the effects were not consistent between FP and TP.

Because of the limited number of markers common to both genetic maps, the bins harboring the QTL were compared to assess the major QTL common to both populations (Fig. 2). Although the QTL for KLEN on chromosome 4, to which Huangzaosi contributed the increasing alleles, were not found in the same or adjacent bins across the two populations, they were very likely a common QTL for KLEN due to the low resolution of the genetic map in this region in Q/H. Furthermore, many QTL with large effects for KVOL, KDEN, KWEI, and GYPP were also clustered in bin 4.05–4.06, in which the increasing alleles were consistently from Huangzaosi. This genomic region seemed critical for grain yield. In addition, the QTL for KDEN and KTHI in bin 1.10 and the QTL for GYPP and KTHI in bin 10.04 in Q/H were also detected in Y/H. The QTL for KLEN in bin 9.02 was specific for Y/H.

The genotypic correlations between predicted and observed testcross performance, $\hat{r}_g$ (M_{FP}, Y_{TP}), were significant ($P < 0.05$) for all traits, with the exception of GYPP in Q/H and KNPP in Y/H (Table 3). The correlation coefficient between $\hat{r}_g$ (FP, TP) and $\hat{r}_g$ (M_{FP}, Y_{TP}) was 0.63 ($P < 0.01$). However, there was no correlation between the

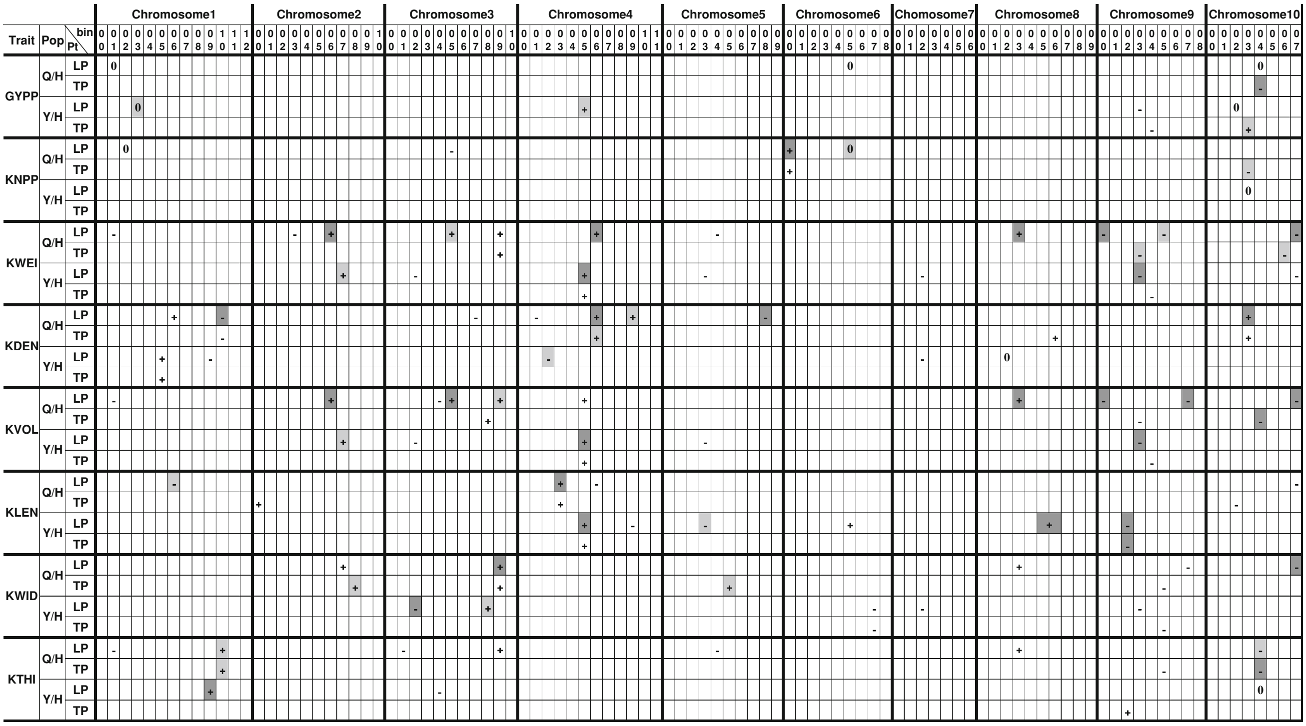

Fig. 2 Chromosomal positions and magnitude of explained phenotypic variance of QTL detected for eight traits evaluated for FP and TP of the two populations. Pop and Pt indicate populations and progeny type, respectively. +, −, and 0 indicate that the effect-increasing allele of the QTL is contributed by Huangzaosi, Qi319/Ye478, or no additive effects detected, respectively

magnitude of $\hat{r}_g$ (M_{FP}, Y_{TP}) and the number of common QTL between FP and TP. For KDEN and KTHI in Y/H, $\hat{r}_g$ (M_{FP}, Y_{TP}) was significant despite of the lack of QTL common to FP and TP.

Discussion

Performances of family per se and testcross progenies

Grain yield is a complex trait and the final outcome of inter-related polygene-controlled developmental processes throughout the plant life cycle (Hallauer and Miranda 1988; Veldboom and Lee 1994; Austin and Lee 1996). It is also the consequence of the net QTL effects at different developmental stages of related traits (Cao and Zhu 2007; Liu et al. 2008). It is of great importance to reveal the genetic relationships between grain yield and its related traits for both family per se and testcross progenies. Knowledge of these relationships contributes to a better understanding of the complex biological basis of grain yield (Guo et al. 2005; Liu et al. 2008). Significant and positive phenotypic and genotypic correlations between GYPP and KNPP, KWEI, KVOL, KLEN, and KWID were found for both types of progeny in the two populations in the present study. Similar correlations for FP were reported by Li et al. (2009), Messmer et al. (2009), and Peng et al. (2011). Therefore, the selection on KNPP, KWEI, KVOL, KLEN, and KWID could be emphasized in not only FP, but also TP. Meanwhile, the selection of KDEN and KTHI within a population should be performed with caution because the direction of their correlation with GYPP was inconsistent across different types of progeny.

Correlations between FP and TP

Varied $\hat{r}_g$ (FP, TP) for the eight yield-related traits were found to be determined by self generations, traits, the choice of the tester, or the combined action of these factors. Our results showed that the ratio of heterozygosity for different $F_{2:3}$ families ranged from 30.7 to 77.5 % in Q/H-FP and from 33.5 to 79.2 % in Y/H-FP. Significant correlations with the ratio of heterozygosity ($r = 0.26$–0.37, $P < 0.01$) in the two populations were consistently observed for only the two highly heterotic traits (i.e., GYPP and KNPP), which could partly explain the low $\hat{r}_g$ (FP, TP) values for GYPP and KNPP. In maize, different levels of heterozygosity affected FP, but not TP, resulting in reduced $\hat{r}_g$ (FP, TP) for heterotic traits in the early selfing generation (Mihaljevic et al. 2005). However, in advanced selfing generations, low $\hat{r}_g$ (FP, TP) values for grain yield were probably caused by recessive genes the effects of which in homozygotes are detrimental (Genter and Alexander 1966).

Table 4 Summary of QTL affecting grain yield and related traits detected with family per se (FP) and their testcross performance (TP) using the unrelated tester Mo17 in Q/H across three environments

Trait	Bin[a]	FP							TP					
		Position	LOD score	R^2_{adj} (%)	p^{b}	A[c]	D[d]	p^{e}	Position	LOD score	R^2_{adj} (%)	p^{b}	A	p^{e}
GYPP	1.01	56	3.71	5.3			9.71							
	6.05	104	3.63	5.6			6.92							
	10.04	94	4.11	7.9			8.17		104	4.11	13.0		−22.04	
	Final fit			18.3	31.7			6.8			12.2	18.7		7.4
KNPP	1.02	60	2.82	4.2			31.81							
	3.05	204	3.11	6.2		−19.83								
	6.00	16	4.46	10.1		26.89			2	2.91	5.2		40.10	
	6.05	104	5.20	9.9			35.30							
	10.03								82	3.69	8.6		−51.55	
	Final fit			22.2	37.5			5.8			10.4	15.7		5.0
KWEI	1.01	34	4.38	5.5		−0.65								
	2.03	116	2.62	5.7		−0.77								
	2.06	230	9.97	17.4		1.49								
	3.05	194	6.31	9.5		1.10								
	3.09	290	3.38	8.0		0.74			300	4.86	6.5		1.90	
	4.06	124	9.63	17.2		1.33								
	5.04	154	4.42	5.5		−0.62								
	8.03	72	7.30	13.4		1.01								
	9.00	20	8.71	14.7		−1.30								
	9.03								68	4.05	9.6		−2.17	
	9.05	106	6.00	9.4		−0.90								
	10.06								172	5.04	9.5		−2.54	
	10.07	210	4.34	14.9		−1.22								
	Final fit			52.8	66.4			41.6			19.5	31.2		11.3
KDEN	1.06	148	5.67	5.2		0.01								
	1.10	234	6.56	22.6		−0.02			236	4.07	5.9		−0.02	
	3.07	246	7.88	6.7		−0.01								
	4.01	78	3.59	5.2		−0.01								
	4.06	132	6.42	10.9		0.02			142	4.51	9.1		0.02	
	4.09	232	5.41	8.4		0.01								
	5.08	264	5.72	10.1		−0.02								
	8.06								134	3.26	7.8		0.02	
	10.03	54	10.96	17.3		0.02								
	10.03								76	4.25	7.1		0.02	
	Final fit			44.9	59.1			34.9			18.8	31.4		4.1
KVOL	1.01	40	5.00	5.7		−0.54								
	2.06	230	9.29	13.9		0.95								
	3.04	58	3.87	7.9		−0.86								
	3.05	198	7.75	14.3		1.25								
	3.08–3.09	290	7.27	8.3		0.64			274	3.64	6.8		1.54	
	4.05	122	5.40	7.7		0.71								
	8.03	72	8.83	14.5		0.91								
	9.00	12	8.44	13.4		−0.91								
	9.03								68	5.04	7.8		−1.68	
	9.07	168	4.41	12.0		−0.89								
	10.04								110	3.26	12.5		−2.34	

Table 4 continued

Trait	Bin[a]	FP							TP					
		Position	LOD score	R^2_{adj} (%)	p^{b}	A[c]	D[d]	p^{e}	Position	LOD score	R^2_{adj} (%)	p^{b}	A	p^{e}
	10.07	212	7.52	21.7		−1.31								
	Final fit			52.3	64.3			43.5			20.7	30.2		6.0
KLEN	1.06	200	5.25	8.9		−0.16								
	2.00								0	4.21	5.7		0.35	
	4.03	106	4.23	12.7		0.25			98	5.95	7.1		0.36	
	4.06	146	2.74	7.8		−0.17								
	10.02								28	3.61	7.6		−0.35	
	10.07	222	7.76	15.9		−0.24								
	Final fit			29.4	38.6			16.2			16.8	21.6		4.5
KWID	2.07	258	5.84	6.1		0.14								
	2.08								302	3.90	9.9		0.29	
	3.09	296	8.26	11.3		0.17			288	3.30	5.5		0.20	
	5.05								180	4.73	9.2		0.26	
	8.03	74	3.91	4.9		0.10								
	9.05								82	2.55	7.2		−0.22	
	9.07	166	4.32	6.7		−0.13								
	10.07	224	21.51	29.0		−0.32								
	Final fit			36.0	44.1			34.3			23.4	33.3		11.1
KTHI	1.01	28	2.92	4.7		−0.08								
	1.10	252	5.97	9.4		0.12			248	3.66	8.1		0.17	
	3.01	16	4.09	5.7		−0.09								
	3.09	288	5.80	7.2		0.09								
	5.04	144	5.06	7.2		−0.09								
	8.03	74	4.95	5.5		0.08								
	9.05								118	4.65	7.7		−0.15	
	10.04	92	6.43	8.5		−0.10			94	3.11	10.9		−0.18	
	Final fit			30.9	40.6			15.4			21.6	30.0		13.1

[a] Bin assignment of QTL is performed based on bin assignment of flanking markers in the IBM2 2008 neighbors reference map obtained from MaizeGDB

[b] Proportion of the genotypic variance explained by detected QTL, calculated as R^2_{adj}/heritability in the whole data set

[c] Additive effect of the QTL calculated in the whole data set. In the $F_{2:3}$ families, QTL effects were associated with the Huangzaosi allele (due to replacement of the Qi319/Ye478 alleles by the Huangzaosi allele). In the testcross populations, QTL effects were estimated by the heterozygote (Huangzaosi/Mo17)—the heterozygote (Qi319/Mo17 or Ye478/Mo17)

[d] Dominant effect of QTL was calculated in the whole data set

[e] Proportion of the genotypic variance explained by detected QTL calculated as R^2_{adj}/heritability in 200 cross-validation runs

Although previous studies reported $\hat{r}_{\mathrm{g}}$ (FP, TP) for agronomically important traits in maize, only a few included kernel-related traits. In the present study, for KWEI, KDEN, KVOL, KLEN, KWID, and KTHI with higher heritability and mainly controlled by additive gene actions, the $\hat{r}_{\mathrm{g}}$ (FP, TP) values were intermediate. For GYPP and KNPP with lower heritability and controlled primarily by non-additive gene actions, the $\hat{r}_{\mathrm{g}}$ (FP, TP) values were low. Mihaljevic et al. (2005) reported that the magnitude of $\hat{r}_{\mathrm{g}}$ (FP, TP) was low to intermediate for grain yield (0.28–0.56) across four crosses and was intermediate to high for traits with higher heritability and presumably mainly additive gene actions, such as grain moisture, kernel weight, protein concentration, and plant height (0.52–0.87). Bekavac et al. (2008) also demonstrated that $\hat{r}_{\mathrm{g}}$ (FP, TP) values were intermediate for highly heterotic traits such as grain yield (0.396–0.592) and high for traits controlled mainly by additive gene actions such as stay green, anthesis-silking interval, stalk water content, and grain moisture (>0.796) in two maize crosses. Thus, effective

Table 5 Summary of QTL affecting grain yield and related traits detected with family per se (FP) and their testcross performance (TP) using the unrelated tester Mo17 in Y/H across three environments

Trait	Bin	FP							TP					
		Position	LOD score	R^2_{adj} (%)	*p*	A	D	*p*	Position	LOD score	R^2_{adj} (%)	*p*	A	*p*
GYPP	1.03	120	5.34	8.8			7.82							
	4.05	182	3.99	8.8		5.35								
	9.03–9.04	124	3.61	7.0		−4.84			136	3.01	5.8		−11.93	
	10.02	76	2.52	3.6			5.46							
	10.03								138	4.75	8.7		12.33	
	Final fit			22.0	39.8			12.0			12.2	24.7		8.3
KNPP	10.03	192	4.37	3.6			18.55							
	Final fit			3.2	6.5			0.0						
KWEI	2.07	196	6.34	8.4		1.04								
	3.02	194	5.39	7.6		−1.10								
	4.05	184	8.88	11.4		1.26			178	5.61	7.9		2.14	
	5.03	12	3.15	6.3		−0.87								
	7.02	8	4.94	7.8		−0.98								
	9.03	104	5.28	11.1		−1.25								
	9.04								134	4.45	7.2		−2.07	
	10.07	318	3.97	5.2		−0.89								
	Final fit			34.3	41.5			19.3			11.9	17.2		3.8
KDEN	1.05	180	3.96	7.6		0.01								
	1.05								214	3.37	6.0		0.02	
	1.09	410	4.45	5.2		−0.01								
	4.02	126	5.73	10.0		−0.01								
	7.02	16	3.52	7.0		−0.01								
	8.02	0	3.21	4.3			0.01							
	Final fit			24.1	52.0			12.1			5.2	10.8		0.0
KVOL	2.07	194	8.24	8.9		1.01								
	3.02	200	7.35	7.0		−0.89								
	4.05	184	10.80	11.9		1.18			178	5.33	7.3		0.87	
	5.03	10	4.31	6.5		−0.80								
	9.03	106	5.79	11.6		−1.16								
	9.04								130	4.67	7.4		−0.91	
	Final fit			31.2	36.8			20.2			11.6	16.7		3.6
KLEN	4.05	192	11.67	17.2		0.34			178	3.61	5.3		0.28	
	4.09	304	4.66	6.8		−0.20								
	5.03	14	3.91	10.0		−0.21								
	6.05	174	3.52	5.3		0.15								
	8.05–8.06	150	5.51	10.7		0.28								
	9.02	82	5.23	11.1		−0.28			62	4.57	16.9		−0.62	
	Final fit			38.4	47.4			27.0			20.0	29.9		22.0
KWID	3.02	196	4.48	10.5		−0.21								
	3.08	294	8.20	8.4		0.18								
	6.07	222	3.15	6.1		−0.16			226	3.24	5.5		−0.25	
	7.02	14	6.93	7.8		−0.19								
	9.03	114	3.60	5.3		−0.14								
	9.05								164	5.72	8.0		−0.30	
	Final fit			25.5	32.2			12.8			11.1	16.6		3.5

Table 5 continued

Trait	Bin	FP							TP					
		Position	LOD score	R^2_{adj} (%)	p	A	D	p	Position	LOD score	R^2_{adj} (%)	p	A	p
KTHI	1.09	410	7.61	15.3		0.15								
	3.04	202	4.96	6.6		−0.09								
	9.02								28	3.86	4.5		0.14	
	10.04	196	5.92	6.8			−0.13							
	Final fit			23.2	31.3			17.0			3.7	5.6		0.0

selection based on FP to improve TP might be achieved only for traits with high heritability rather than grain yield and kernel number per plant.

The tester's genetic constitution also affected the $\hat{r}_g$ (FP, TP) values. In the present study, the genetic variances for FP of all traits, with the exception of GYPP and KNPP in the two populations and KLEN in Q/H, were higher than those for TP. The dominant alleles fixed in the tester masked the effect of the segregating alleles at many loci and resulted in a reduced genotypic variance for TP compared with FP, which eventually led to decreased $\hat{r}_g$ (FP, TP) (Smith 1986; Austin et al. 2000; Mihaljevic et al. 2005; Bekavac et al. 2008; Frascaroli et al. 2009). Some other factors, such as epistasis, also affect the ratio of σ^2_g for LP and TP, and $\hat{r}_g$ (LP, TP) may be the reason for the higher genetic variances for TP compared with those for LP (Smith 1986; Mihaljevic et al. 2005; You et al. 2006). In addition, Mihaljevic (2005) suggested the importance of identification of QTL with TP using an unrelated tester inbred, which corresponds to the testing situation in hybrid breeding programs. From the standpoint of modern maize-breeding practices, the genetically unrelated tester "Mo17" was used in this study. Thus, the results obtained here may be of benefit to breeders and are suitable for application to contemporary maize breeding programs.

CV

Because of the limitation of available statistical methods, the estimation of individual QTL effects and the proportion of genotypic variance explained by QTL (*p*) were severely inflated in previous studies, which led to overly optimistic assessments of the prospective of MAS (Utz et al. 2000).

To obtain an unbiased estimation of *p* and a realistic assessment of the prospects of MAS, an analysis of CV was carried out, as recommended by Utz et al. (2000). The genotypic variance explained in the CV analysis is much lower than that in the whole data set for all of the traits, suggesting a large upward bias in estimation of genotypic variance by the model. $p_{\mathrm{TS.ES}}$ for all of the traits across both types of progenies was <50.0 %, and $p_{\mathrm{TS.ES}}$ was on average 43 and 27 % of p_{DS} for FP and TP across the two populations, respectively. Rodríguez et al. (2008) detected two QTL for leaf color that explained 28.2 % of genotypic variance estimated from the whole data set. However, they explained only 3.7 % of the genotypic variance in CV analysis. Ordas et al. (2009) found that the final fit for detected QTL explained 48.1, 8.3, and 19.9 % of the genotypic variance for the date of silking, kernel damage, and stalk tunnel, respectively. However, these QTL explained only 0.7, 0.0, and 6.5 % of genotypic variance in 200 CV runs, respectively. The inflation of p_{DS} compared with $p_{\mathrm{TS.ES}}$ was caused by genotypic sampling (Bohn et al. 2001). To obtain reliable and high $p_{\mathrm{TS.ES}}$ values for quantitative traits and a relatively high efficiency of MAS over conventional phenotypic selection, a large population size (Utz et al. 2000; Bohn et al. 2001; Schön et al. 2004; Buckler et al. 2009), a model containing complex epistasis (Carlborg and Haley 2004; Francia et al. 2005; Mihaljevic et al. 2005), and high heritability by increasing the number of replications and environments (Hallauer and Miranda 1988) should be utilized. When a large number of QTL were transferred or when additional QTL for a trait were still undetected, phenotypic selection combined with MAS should be adopted (Collard and Mackill 2008). This approach had advantages over phenotypic screening or MAS alone in terms of maximizing genetic gain (Bohn et al. 2001), especially when large population sizes were used and trait heritability was low.

Comparison of QTL between FP and TP

In our study, about 50–75 % of the major QTL were specific for FP or TP. Similar results were obtained by Mihaljevic et al. (2005), who found that approximately 38–75 % QTL detected with inbred progeny for four agronomic traits of four populations were not detected with testcross progeny, while 50–75 % of QTL detected with testcross progeny were not associated with those QTL detected with inbred progeny. The discrepancies between the QTL with FP and the QTL with TP could be attributed to the following: (1) The additive effects of the QTL detected with the TC progenies actually represented the differential intra-locus interactions between the parental alleles of the $F_{2:3}$ families and the tester allele or the difference between the two heterozygotes (Huangzaosi/tester–Qi319/tester) or (Huangzaosi/tester–Ye478/tester), where the tester was Mo17. Possible interactions between the parental allele and the tester allele should also be considered when comparing different types of progeny (Austin et al. 2000; Mihaljevic et al. 2005; You et al. 2006). Thus, if the tester allele was dominant to the parental alleles, no QTLs would be detectable (Mei et al. 2003; Papst et al. 2004; You et al. 2006). (2) Other probable reasons include the power of QTL detection with FP and TP, which was influenced by the statistical model used in QTL analysis (Schön et al. 2004; Mihaljevic et al. 2005); some minor-effect QTL that were undetected due to the limited population size (Utz et al. 2000; Papst et al. 2004; Schön et al. 2004; Mihaljevic et al. 2005); QTL × environment interactions (Utz et al. 2000; Peng et al. 2011); epistatic effects that were too complicated to be understood using the available bioinformatics tools; and the limited population size (Carlborg and Haley 2004; Mihaljevic et al. 2005; Holland 2007; Peng et al. 2011).

QTL with congruent positions have been enumerated in many similar studies but yielded no information about the conformity of QTL effects. The genetic correlation between the predicted and observed phenotypic values, $\hat{r}_g$ (M_{FP}, Y_{TP}), estimated the QTL congruency considering both the positions and effects of QTL. In the present study, $\hat{r}_g$ (FP, TP) significantly correlated with $\hat{r}_g$ (M_{FP}, Y_{TP}), but was not significantly correlated with the number of common QTL. Thus, $\hat{r}_g$ (M_{FP}, Y_{TP}) was more suitable for assessment of the potential for indirect improvement of TP based on the QTL detected with FP than was counting the number of common QTL (Mihaljevic et al. 2004, 2005).

Common QTL between FP and TP and positional convergence of yield-related QTL in maize

In this study, a total of 123 QTL distributed among ten chromosomes were detected for eight traits across both types of progeny in the two populations. Some QTL common to both FP and TP that accounted for the large phenotypic variance were clustered in four chromosomal regions (bin 1.10, 4.05–4.06, 9.02, and 10.04). The publicly available QTL database (http://www.maizegdb.org) was subsequently explored to search their alignment QTL identified in the same genomic regions in previous studies.

The allele contributed by Huangzaosi in bin 1.10 caused a decrease in KDEN and an increase in KTHI. The QTL was also reportedly associated with starch yield, protein content, kernel weight, kernel number, grain moisture, and grain yield in five populations: UHoh KW1265/D146 F_2 and F_3 (Schön et al. 1994; Lübberstedt et al. 1997; Melchinger et al. 1998), UIUC W6786/IL731A F_2 (Azanza et al. 1996), CIMMYT Ac7729/Ac7643 F_2 (Ribaut et al. 1997), and INRA Io/$F2$ RIL (Damerval et al. 1994). The common genetic control of yield-related and seed-quality traits at this locus could be partly explained by the close correlations among KDEN, seed quality, and yield-related traits at the phenotypic level (Zhang et al. 2007; Peng et al. 2011).

For the QTL associated with KLEN in bin 4.05–4.06, Huangzaosi conferred the effect-increasing allele at this locus across, suggesting that the genomic region on chromosome 4 may play important roles in conferring yield advantages in the Tangsipingtou heterotic group. Clusters of the QTL for KVOL, KDEN, KWEI, and GYPP at this locus suggested a common genetic control of the traits through close linkage or pleiotropy. The QTL at the loci for kernel weight, grain yield, ear diameter, kernel row number, and protein concentration in five populations, including UMN chapalote/*Z. mexicana* F_2 (Doebley et al. 1994), Bergamo B73/A7 F_3 (Ajmone-Marsan et al. 1995, 2001), ISU Mo17/H99 F_3 and RIL (Veldboom and Lee 1994; Austin and Lee 1996), and INRA Io/$F2$ RIL (Damerval et al. 1994) have been reported. To assess the precision of the determined QTL locations on chromosome 4, the QTL frequency distribution was used to determine the most likely QTL position based on 1,000 CV/G runs (Utz et al. 2000) (Fig. 3). The QTL frequency distributions for the target traits were in good agreement with LOD curves obtained with CIM for the whole data set. The QTL for KLEN-FP (the frequency of QTL detection was 78.7 %), KLEN-TP (65.5 %), KVOL-FP (94.6 %), KDEN-FP (93.1 %), and KWEI-FP (99.2 %) in Q/H and for KLEN-FP (98.5 %), KVOL-FP (88.3 %), KVOL-TP (68.0 %), KWEI-FP (67.3 %), KWEI-TP (74.5 %), and GYPP-FP (75.6 %) in Y/H were confirmed in >60 % of the CV runs, and clear peaks for the QTL frequency distribution in bin 4.05–4.06 were observed. Hence, they corroborated the validity of identification.

The QTL for KLEN in bin 9.02 were also found to be associated with yield-related traits in previous studies, such as kernel row length, kernel number per 10 plants, kernel weight, ear length, and ear diameter in four populations [i.e., B73/Mo17 F_2 (Beavis et al. 1994), CIMMYT Ac7729/Ac7643 F_2 (Ribaut et al. 1997), CIMMYT Ki3/CML139 F_2 (Khairallah et al. 1998), and ISU Mo17/H99 RIL (Austin and Lee 1998)].

The QTL for KTHI and GYPP in bin 10.04 were also found to be associated with yield-related traits, including ear diameter, grain yield, grain moisture, starch yield in seven populations [i.e., ISU Mo17/H99 RIL (Austin and Lee 1996, 1998), Bergamo B73/A7 F_2 and F_3

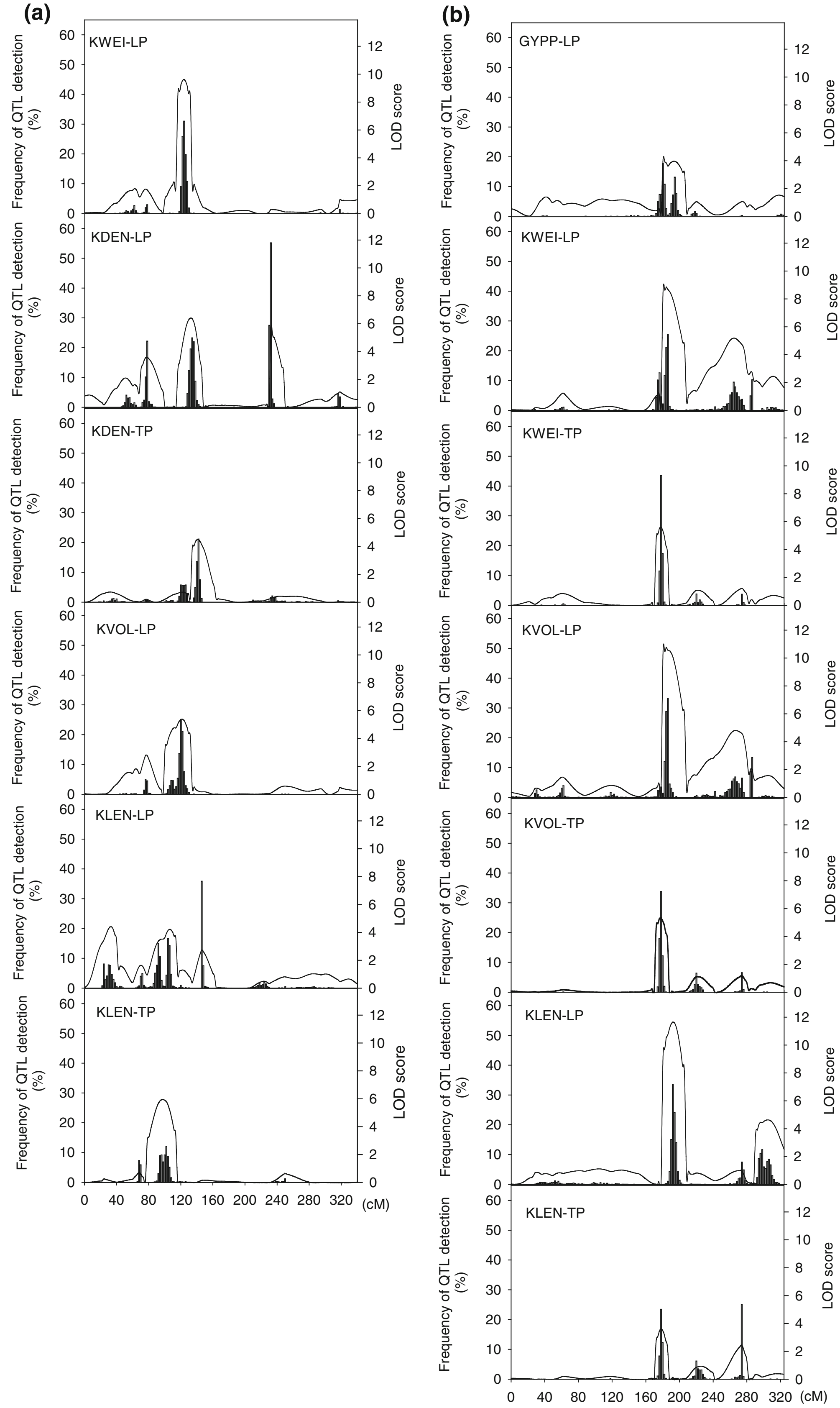

Fig. 3 Frequency distribution of QTL for eight traits with 2-cM intervals on chromosome 4 obtained from 1,000 CV runs in Q/H (**a**) and Y/H (**b**). The *solid lines* indicate the LOD curves determined from the entire data set using composite interval mapping

(Ajmone-Marsan et al. 1995, 1996), B73/G79 F_3 (Agrama et al. 1999), CIMMYT Ac7643S5/(Ac7729/TZSRWS5) $F_{3:4}$ (Ribaut et al. 1997), and UHoh KW1265/D146 F_2 and F_3 (Lübberstedt et al. 1997; Melchinger et al. 1998)].

Four common QTL with large effects were identified in this study, and were detected in a wider range of genetic backgrounds and environments in maize, suggesting that some major genes are conserved across different materials in maize; thus these are valuable for further maize gene cloning and breeding. Single large-scale marker-assisted selection (SLS–MAS) was particularly suitable for pyramiding favorable alleles at few major QTL at an early generation (such as F_2 or F_3 generations) derived from elite parents, which eliminated undesirable gene combinations and fixed/enriched favorable alleles as early as possible, allowing breeders to focus on the most promising materials in subsequent generations (Babu et al. 2004; Collard and Mackill 2008). Furthermore, use of marker-assisted backcross (MABC) selection, Ribaut and Ragot (2007) facilitated successful transfer of the favorable alleles of five major QTL to improve the grain yield of tropical maize under drought conditions. Incorporating these major loci into an elite germplasm by MABC was also an efficient way to develop improved inbred lines.

Implications for genetic improvement and marker-assisted selection in maize hybrid breeding

The magnitude of genotypic correlations between FP and TP was crucial for simultaneous improvement of inbred lines and hybrids. For traits with prevailing additive effects and relatively high $\hat{r}_g$ (FP, TP) and $\hat{r}_g$ (M_{FP}, Y_{TP}) values, such as KWEI, KVOL, KLEN, and KWID, effective selection based on FP to improve TP could be achieved. However, low values of $\hat{r}_g$ (FP, TP) for grain yield showed that the ultimate use of inbred lines in hybrid combinations must be evaluated in TP. Furthermore, KWEI, KVOL, KLEN, and KWID—which have high heritability—were significantly correlated with grain yield. Thus, the selection for KWEI, KVOL, KLEN, and KWID could efficiently contribute to high and stable GYPP.

Our QTL analysis had several implications for hybrid maize breeding. First, the CV analysis showed that a large population size, a complex statistical model, and more replications and environments should be considered to obtain a high proportion of the genotypic variance explained by the detected QTL and a relatively high efficiency of MAS. Second, QTL common to FP and TP with large effects located in bins 1.10, 4.05–4.06, 9.02, and 10.04, which were detected in much wider ranges of genetic backgrounds and environments, were valuable loci for improving the grain yields of inbreds and hybrids. Finally, Qi319, Ye478, Huangzaosi, and Mo17 are foundation inbred lines widely used in maize-breeding programs in China. Many currently and historically important maize single-cross hybrids were developed using two of these four lines or their derived descendents (Ci et al. 2011; Li et al. 2011; Wang et al. 2011). The results of QTL mapping we report here could provide a guide to the development of single hybrids and thus facilitate further exploitation of the heterotic patterns of non-Lancaster × Lancaster (especially for Tangsipingtou × Lancaster).

Acknowledgments This work was partly supported by grants provided by the Ministry of Science and Technology of China (2011CB100100, 2009CB118401, 2011DFA30450), Natural Science Foundation of China (U1138304) and Foundation of President of The Tianjin Academy of Agricultural Sciences (11001). We thank Dr. Matthias Frisch and two anonymous reviewers for valuable suggestions and careful corrections. We are grateful to Prof. H Friedrich Utz and Albrecht E Melchinger of University of Hohenheim for providing the PLABSTAT and PLABQTL software and their excellent advices in the data analysis, and to Prof. Xianchun Xia of Chinese Academy of Agriculture Sciences, Prof. Shoucai Wang of Chinese Agricultural University, Prof. Martin Bohn of University of Illinois at Urbana-Champaign, Prof. Edward Buckler of Cornell University for advices on data analysis and discussion, to Prof. Jiankang Wang and Dr. Huihui Li of Chinese Academy of Agriculture Sciences and Prof. Chenwu Xu of Yangzhou University for advices on data analysis.

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