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ORIGINAL PAPER

QTL analysis for yield components and kernel-related traits in maize across multi-environments

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Abstract Huangzaosi, Qi319, and Ye478 are foundation inbred lines widely used in maize breeding in China. To elucidate genetic base of yield components and kernel-related traits in these elite lines, two $F_{2:3}$ populations derived from crosses Qi319 × Huangzaosi (Q/H, 230 families) and Ye478 × Huangzaosi (Y/H, 235 families), as well as their parents were evaluated in six environments including Henan, Beijing, and Xinjiang in 2007 and 2008. Correlation and hypergeometric probability function analyses showed the dependence of yield components on kernel-related traits. Three mapping procedures were used to identify quantitative trait loci (QTL) for each population: (1) analysis for each of the six environments, (2) joint analysis for each of the three locations across 2 years, and (3) joint analysis across all environments. For the eight traits measured, 90, 89, and 58 QTL for Q/H, and 72, 76, and 51 QTL for Y/H were detected by the three QTL mapping procedures, respectively. About 70% of the QTL from Q/H and 90% of the QTL from Y/H did not show significant QTL × environment interactions in the joint analysis across all environments. Most of the QTL for kernel traits exhibited high stability across 2 years at the same location, even across different locations. Seven major QTL detected under at least four environments were identified on chromosomes 1, 4, 6, 7, 9, and 10 in the populations. Moreover, QTL on chr. 1, chr. 4, and chr. 9 were detected in both populations. These chromosomal regions could be targets for marker-assisted selection, fine mapping, and map-based cloning in maize.

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Introduction

Grain yield is the most important trait in maize breeding programs and it is also one of the most complex quantitative traits (Austin and Lee 1996), determined by several yield components, e.g. number of plants per hectare, kernel number per plant, and kernel weight. Yield components always have higher heritability than grain yield (Austin and Lee 1996; Messmer et al. 2009) and improvement in grain yield can be achieved by increasing yield components (Gupta et al. 2006). Hence, most efforts have been devoted to understand kernel number and kernel weight. Eco-geographical factors, physiological factors, seed developmental processes, and evolutionary causes affecting kernel size and kernel number have been reviewed recently (Gupta et al. 2006; Sadras 2007; Sadras

and Denison 2009). There is essentially a tradeoff between kernel size and kernel number (Henery and Westoby 2001), which increases the difficulty in the genetic improvement for yield.

The genetic dissection of traits correlated with yield would contribute to the understanding of the complex biological pathway of yield formation and yield improvement (Ribaut et al. 1997; Wen and Zhu 2005). There are several reasons for paying more attention to traits related to kernel size. First, kernel size is a major determinant of kernel weight, which is a component of grain yield (Messmer et al. 2009). Second, kernel size is related to early vigor of maize. Larger kernels usually contribute to high germination in cool humid regions (Revilla et al. 1999). Third, the end use quality of maize (e.g., flour yield and protein content) is influenced by kernel size (Gupta et al. 2006). Kernel size is significantly influenced by kernel shape traits such as kernel length, kernel width, and kernel thickness (Li et al. 2009). Therefore, kernel weight can be dissected into more parameters such as kernel density, kernel volume, kernel length, kernel width, and kernel thickness.

In the past 2 decades, molecular-marker techniques have been widely used to investigate complex quantitative traits in different crop species (Bernardo 2008). Gupta et al. (2006) summarized many major loci and genes involved in regulation of seed size and number in maize as well as in rice, barley, wheat, *Arabidopsis*, pea, and tomato. In some crops, QTL analysis for kernel shape traits were performed, such as wheat (Sun et al. 2009; Breseghello and Sorrells 2007) and rice (Agrama et al. 2007), indicating that kernel shape traits significantly influenced grain yield. A few QTL for kernel-related traits of rice, such as *GS3* (Fan et al. 2006), *GW2* (Song et al. 2007), *GIF1* (Wang et al. 2008), *Qsw5* (Shomura et al. 2008), *GW5* (Weng et al. 2008), etc. were isolated in the recent years. To date, however, mapping QTL for kernel shape in maize is rare. Most of QTL studies in maize focused on grain yield and kernel weight (Melchinger et al. 1998; Ribaut et al. 1997; Schön et al. 1994; Kozumplik et al. 1996; Ajmone-Marsan et al. 1995; Goldman et al. 1993). The QTL related to grain yield and kernel weight were distributed on all ten chromosomes (Schaeffer et al. 2006). However, relationships between kernel-related traits and yield components at the molecular level are still poorly understood (Li et al. 2009).

The complex physiological processes and high sensitivity to environments resulted in low heritability for grain yield (Hallauer and Miranda 1988; Yadav et al. 2003; Rebetzke et al. 2008). A significant change in the ranking of varieties in different environments ("crossover" types) is often caused by genotype × environment interaction (GEI), which is a major challenge to breeders and results in slow genetic progress (Pidgeon et al. 2006). Because it is closely related to the stability of varieties in plant breeding, GEI of quantitative traits has been extensive investigated (Finlay and Wilkinson 1963; Crossa et al. 1990). QTL analysis has made it possible to break down GEI into its constituent QTL × environment interaction (QEI). Therefore, many researchers have developed methodology to identify QTL with significant interactions with the environment such as mixed model (Boer et al. 2007) and factorial regression models (Vargas et al. 2006). Through stepwise joint mapping, Messmer et al. (2009) successfully identified major QTL in response to various environments by mixed-model based composite interval mapping (MCIM) (Wang et al. 1999). It should be pointed out that up to now most QTL identified for yield components and kernel-related traits could explain only a small percentage of phenotypic variation (often less than 10%) and could not be repeated in different environments and populations (Bernier et al. 2008). Many studies identified QTL in two or three environments (Hittalmani et al. 2003), not in a large number of METs (multiple environment trials), which makes the stability of grain yield QTL difficult to evaluate. A better understanding of QEI and QTL by QTL interaction (epistasis) is critical for marker-assisted selection (Lee 1995; Mohan et al. 1997; Messmer et al. 2009; Rebetzke et al. 2008).

The main objectives of this study were to (1) identify QTL for yield components and kernel-related traits across multiple environments and estimate their effects; (2) understand the stability of QTL and their interactions with environments and detect some constitutive QTL; (3) dissect QTL by QTL interaction (epistasis) for these traits; and (4) reveal the relationships between yield components and kernel-related traits at phenotypic and molecular levels. The results obtained in this study could contribute to the development of efficient approaches for fine mapping and maize breeding in the future.

Materials and methods

Plant materials

Qi319, Ye478, and Huangzaosi are foundation inbred lines in China maize breeding and are representatives of P, Reid, Tangsipingtou heterotic groups, respectively (Wang et al., 2008). A great number of commercial single-cross hybrids were developed by two of these three lines or their derived descendents in China. Two $F_{2:3}$ populations, Qi319 × Huangzaosi (hereafter Q/H) and Ye478 × Huangzaosi (Y/H), were developed for QTL mapping. Random F_2 plants derived from the two crosses were self-pollinated to generate $F_{2:3}$ families. Population sizes were 230 for Q/H and 235 for Y/H.

Field experiment

The $F_{2:3}$ families and their parents were evaluated in 2 years (2007 and 2008) and three locations, i.e. 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 with diverse agro-ecological conditions represented three main maize-growing regions in China. Each year/location combination was considered as an environment. Abbreviations were used to identify the different environments, i.e. E1, E2, E3, E4, E5, and E6 indicated 2007Henan, 2008Henan, 2007Beijing, 2008Beijing, 2007Xinjiang and 2008Xinjiang, respectively. The two populations were summer sown in Henan and spring sown in Beijing and Xinjiang. Trials were conducted in randomized complete blocks with two replications. Each plot consisted of a single row, with 4-m length and spaced 0.6-m apart. The plant density was 52,500 plants/ha. Trials were fertilized following local standard practices at each location and were treated with herbicide to avoid weeds.

Phenotypic data were collected for (1) grain yield per plant (GYPP, g), estimated from the average of five plants in the middle of each row; (2) 100-kernel weight (KWEI, g), estimated from the average of three measurements of the weight of 100 randomly selected kernels; (3) kernel number per plant (KNPP), calculated by the formula of KNPP = 100 × GYPP/KWEI; (4) 100-kernel volume (KVOL, ml), estimated from the average of three measurements of the volume of 100 randomly selected kernels; (5) kernel density (KDEN, g/ml), calculated by dividing 100-kernel weight by 100-kernel volume; (6) 10-kernel length (KLEN, cm), estimated from the average of 5 measurements of the length of 10 kernels in the middle of an ear; (7) 10-kernel width (KWID, cm), estimated from the average of 5 measurements of the width of 10 kernels in the middle of an ear; and (8) 10-kernel thickness (KTHI, cm), estimated from the average of 5 measurements of the thickness of 10 kernel in the middle of an ear.

Statistical analysis of phenotypic data

Analysis of variance was conducted by PROC GLM in SAS8.0 (SAS Institute 1996) with genotype, environments, interaction between environments and genotype, and replications as random effects. The broad-sense heritability (h^2) for each trait was calculated on an plot basis as $h^2 = \sigma_g^2/(\sigma_g^2 + \sigma_{ge}^2/e + \sigma_\varepsilon^2/re)$, where σ_g^2, σ_{ge}^2 and σ_ε^2 were estimates of genotypic, genotype environment interaction and experimental error variances, respectively, while e and r were the numbers of environments and replications per environment, respectively (Hallauer and Miranda 1988).

The Pearson's phenotypic correlation coefficients among yield components and kernel-related traits across all environments were calculated on a mean basis using the SAS PROC CORR (SAS Institute 1996). The genetic correlations among traits were conducted with PLABSTAT software (Utz 1997). Principal component analysis (PCA) was carried out for the 230 (Q/H) or 235 (Y/H) lines using the mean values of the eight traits for the six environments. PCA was based on a correlation matrix and presented as biplot ordinations of lines (PC scores). Two components were extracted using eigenvalues >1 for the two populations, respectively.

Maker analysis and genetic map construction

Genomic DNA was extracted from young leaves of $F_{2:3}$ and their parents (15 plants per $F_{2:3}$ line as a bulk) using the CTAB method (Chen and Ronald 1999). A total of 194 and 159 polymorphic SSR primer pairs obtained from http://www.maizegdb.org/ were applied to construct two genetic maps for QTL mapping, respectively, using Mapmaker v3.0 software (Lander et al. 1987). The recombination frequencies were converted to genetic distance using Haldane mapping function. The maps covered all 10 maize chromosomes with a total genome size of 2493.7 and 3168.9 cM. The average marker intervals were 12.9 and 20.1 cM for Q/H and Y/H, respectively. Due to a limited number of common markers, joint map construction for the two populations was not possible. Therefore, QTL mapping was conducted separately for each individual population.

QTL analysis

QTL mapping was performed using QTLNetwork software version 2.0 (Yang et al. 2007), in which a mixed-model based composite interval mapping (MCIM) was included (Wang et al. 1999). For the two populations, three mapping analyses were carried out as follows: (1) analysis for each of the six environments; (2) joint analysis per location, combining data of 2 years for each location; (3) joint analysis across all environments. Mixed-model based composite interval mapping was undertaken by using forward–backward stepwise with threshold of $P = 0.05$ to select cofactors, and window size set at 10 cM. The threshold for declaring the presence of a significant QTL for each trait-environment combination was defined by 1,000 permutations at a significance level of $P = 0.05$. The confidence interval calculated by the odd ratio reduced by a factor 10 was averaged for each of the QTL (Yang et al. 2007). The final genetic model incorporated significant additive, dominance and epistatic effects, as well as their interactions with environments. In each population, QTL detected in different environments for the same trait were

considered to be the same if their confidence intervals overlapped. A QTL was considered to be stable when the QTL × environment interaction was not significant.

The association between QTL for different traits was determined using the hypergeometric probability function (Larsen and Marx 1985) according to Paterson et al. (1995):

$$P = \frac{\binom{l}{m}\binom{n-l}{s-m}}{\binom{n}{s}}$$

where n is the comparable number of intervals (map length divided to average QTL interval of both traits), m is the number of "matches" (QTL of two traits with >50% overlap of their confidence intervals) declared between QTL, l is the total number of QTL for the trait with larger number of QTL and s is the number of QTL for the trait with smaller number of QTL (Peleg et al. 2009).

Results

Phenotypic performance in different environments

The heritability close to or above 0.7 was observed for all of the traits across six environments in the two populations (Table 1). In contrast to the heritability of GYPP and KNPP, the heritability of the other traits was relatively high (more than 0.8), except that of KDEN in Y/H (0.69). The small genotype variance for KDEN in Y/H could be the major reason.

Although the parents showed no significant difference ($P > 0.05$) for GYPP across all environments, the yield components of the parents differed considerably (suppl. Table 1). In general, Huangzaosi showed higher KNPP than the other two parental lines, whereas Qi319 and Ye478 showed larger kernel weight than Huangzaosi.

The lines within each population were significantly ($P < 0.001$) different for all traits (Table 1). Significant ($P < 0.001$) genotype × environment interactions for most traits in the two populations were also observed, with the exception of those for KDEN and KTHI in Y/H. It indicated that the performance of the two populations for most traits across environments was quite different. The segregation in each population for GYPP was consistent across environments (Fig. 1). The phenotypic values of the progenies for GYPP exhibited obvious transgressive segregation in each population, indicating polygenic characteristics of GYPP. But the mean GYPP varied considerably across all environments with the range from 52.6 to 76.7 g and 53.1 to 108.3 g in Q/H and Y/H, respectively. Both of the populations consistently showed that the order of mean GYPP from highest to lowest was Xinjiang, Beijing, and Henan. The mean GYPP in Xinjiang was more than 40% higher than that in Henan in the two populations (Fig. 1).

Correlation analysis

The phenotypic and genetic correlations among yield components and kernel-related traits across all environments were listed in Table 2. Basically, GYPP had significant positive correlations with two yield components, i.e. KNPP and KWEI. For Q/H, GYPP also had significant correlations with other traits involved while for Y/H, the

Table 1 Analysis of variance (ANOVA) for yield components and kernel-related traits for the two populations (Q/H and Y/H) in six environments

Source of variation	Mean square							
	GYPP	KNPP	KWEI	KDEN	KVOL	KLEN	KWID	KTHI
Genotype (G)	2,003.2***/ 1,586.3***	28,077.0***/ 16,855.1***	72.2***/ 74.0***	0.014***/ 0.0068***	51.1***/ 55.6***	1.66***/ 3.10***	1.57***/ 1.97***	0.75***/ 0.62***
Environment (E)	47,409.5***/ 152,393.1***	352,148.0***/ 1,239,422.0***	2,952.4***/ 4,374.5***	0.21***/ 0.18***	2,161.0***/ 3,248.5***	50.1***/ 92.5***	10.7***/ 25.3***	28.3***/ 6.13***
G × E	506.2***/ 470.0***	7,347.9***/ 5,471.8***	9.40***/ 8.29***	0.0027***/ 0.0021	5.70***/ 5.41***	0.27***/ 0.35***	0.18***/ 0.23***	0.12***/ 0.090
Block	234.7/98.6	1,631.8/19,102.8*	82.5***/ 82.4***	0.021**/ 0.0020	32.1**/ 56.1***	2.12**/ 0.12	0.93*/ 0.054	0.0001/ 0.13
Error	271.7/298.9	4,252.8/3,920.7	5.20/5.33	0.0021/ 0.0020	3.48/3.48	0.20/0.26	0.14/0.19	0.090/ 0.088
Heritability (h^2)	0.75/0.70	0.74/0.68	0.87/0.89	0.81/0.69	0.89/0.90	0.83/0.89	0.88/0.88	0.84/0.85

*, ** and *** Significance at $P < 0.05$, 0.01 and 0.001, respectively

The values in front of "/" indicate mean square for Q/H, the values behind of "/" indicate mean square for Y/H

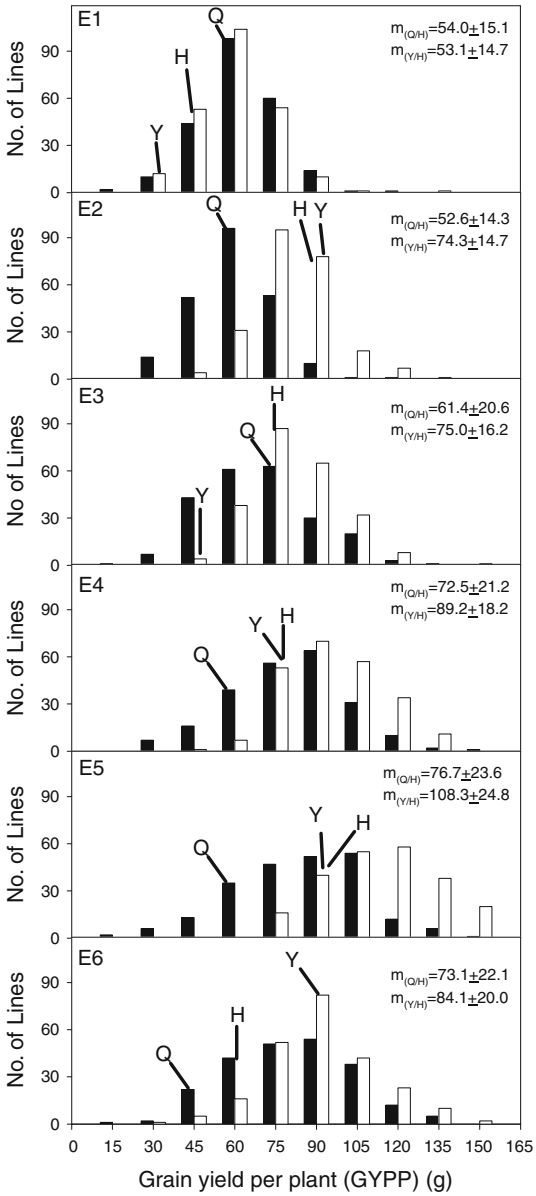

Fig. 1 Frequency distributions of grain yield per plant (GYPP) for the two $F_{2:3}$ populations (Q/H and Y/H) in six environments. The *black* and *white* histograms show the phenotypic distributions of GYPP for Q/H and Y/H, respectively. Means for Qi319 (Q), Ye478 (Y), and Huangzaosi (H) are labeled. Mean and standard errors of each population in each environment are reported

phenotypic correlations between GYPP and KDEN/KTHI were not significant. In addition, the results indicated that the correlation between the two yield components might be different in the two populations because there was a significant negative correlation between KNPP and KWEI in Y/H, while KNPP did not correlate significantly with KWEI in Q/H.

Higher phenotypic and genetic correlations between KVOL and KWEI, KWID and KVOL, and KWID and KWEI in both of the two populations were observed. Although KDEN associated with KWEI in Q/H, it appeared not to be very important for KWEI in Y/H. It was worthwhile to note that the correlation coefficient between GYPP and KLEN was significant and KLEN positively associated with KNPP and KWEI in the two populations. Furthermore, the present study showed that a linear fit was significant between GYPP and KLEN (Fig. 2). The slope of regression in each environment ranged from 18 to 27 in Q/H and 10 to 22 in Y/H, respectively. It indicated that although there were considerable differences in irradiance, temperature, precipitation, air relative humidity, and planting date in different eco-geographical locations and years, the linear regression relationship was very robust for different environments.

Two major principal components (eigenvalues >1) that collectively explained 75.8 and 71.4% of the variance were extracted for Q/H and Y/H in the PCA, respectively (Fig. 3). For Q/H (Fig. 3a), principal component 1 (PC1) explained 45.0% of the variance and was positively associated with KVOL, KWEI, KWID, KLEN, GYPP, and KTHI. PC2 explained 30.8% of the variance and was positively associated with KNPP, GYPP, KLEN, and KDEN and was negatively associated with KTHI. For Y/H (Fig. 3b), PC1 explained 45.8% of the variance and was positively associated with KVOL, KWEI, KWID, KLEN, KTHI and GYPP. PC2 explained 25.6% of the variance and was positively associated with KNPP GYPP, and KLEN and negatively associated with KTHI. These results were supported by correlation analysis (Table 2).

QTL detected by using three mapping procedures

A total of 90 and 72 QTL for all eight traits were detected in Q/H and Y/H, respectively, based on single environment analysis (Table 3). Most QTL detected by the single environment analysis were consistent in direction but varied in magnitude across all environments in the two populations, with an exception of *Yqkwei9,* which had opposite additive effects in two environments (suppl. Table 3). Some important QTL clusters were found on chr. 1, chr. 6, and chr. 10 in Q/H, and on chr. 1, chr. 4, chr. 7, and chr. 9 in Y/H (Figs. 4a, 5a). When only major QTL (QTL with $R^2 > 10\%$ in at least one environment and also detected based on joint analysis across all six environments) or QTL detected in at least two environments were considered, both of the male and female parents contributed the effect-increasing allele for most traits in the two populations (suppl. Table 2, 3), with the exception of KTHI with the increasing alleles derived from Qi319 at all loci in Q/H; KWID with the increasing allele derived from Ye478 at only one locus, and KNPP and KDEN with no additive effect at all loci in Y/H.

Table 2 Phenotypic (upper value) and genetic (lower value) correlations among yield components and kernel-related traits in Q/H (right diagonal) and Y/H (left diagonal) across six environments

	GYPP	KNPP	KWEI	KDEN	KVOL	KLEN	KWID	KTHI
GYPP	1	0.85**	0.48**	0.33**	0.41**	0.62**	0.30**	−0.21**
		0.83**	0.51**	0.36**	0.44**	0.65**	0.32**	−0.21**
KNPP	0.76**	1	−0.03^{ns}	0.19**	−0.08^{ns}	0.42**	−0.12^{ns}	−0.51**
	0.71**		−0.05^{ns}	0.22**	−0.11*	0.42**	−0.16*	−0.55**
KWEI	0.47**	−0.19**	1	0.34**	0.96**	0.51**	0.78**	0.46**
	0.51**	−0.25**		0.31**	0.96**	0.51**	0.82**	0.51**
KDEN	−0.03^{ns}	−0.03^{ns}	−0.03^{ns}	1	0.06^{ns}	0.06^{ns}	−0.13^{ns}	−0.23**
	−0.12*	−0.07^{ns}	−0.11*		0.04^{ns}	0.02^{ns}	−0.17**	−0.29**
KVOL	0.46**	−0.18**	0.98**	−0.23**	1	0.53**	0.87**	0.56**
	0.51**	−0.22**	0.98**	−0.28**		0.54**	0.91**	0.61**
KLEN	0.56**	0.25**	0.54**	−0.35**	0.60**	1	0.39**	−0.12^{ns}
	0.58**	0.22**	0.55**	−0.46**	0.62**		0.40**	−0.11*
KWID	0.32**	−0.21**	0.78**	−0.05^{ns}	0.77**	0.25**	1	0.50**
	0.34**	−0.29**	0.82**	−0.08^{ns}	0.80**	0.22**		0.56**
KTHI	−0.01^{ns}	−0.41**	0.56**	−0.28**	0.60**	0.11^{ns}	0.39**	1
	0.03^{ns}	−0.47**	0.61**	−0.35**	0.66**	0.13*	0.41**	

*, ** and ^{ns} indicate significance at $P <$ 0.05, 0.01 and non-significant effect, respectively

The joint QTL analysis per location revealed 89 and 76 QTL for eight traits in Q/H and Y/H, respectively, being almost equal to QTL number detected based on single environment analysis. Similar to the analysis of single environment QTL, more QTL were detected by the joint analysis in Henan than those in other two locations in Q/H, but not in Y/H (Table 3). Of all QTL, only three in Q/H and two in Y/H showed significant QEI over years in a single location (Table 3; Figs. 4b, 5b). All of the QTL that interacted with environments were detected in Xinjiang in the two populations. For kernel shape traits, all of the QTL were stable across years at the same locations, with the exception of one QTL for KTHI in Y/H.

The joint analysis across all environments revealed 58 QTL in Q/H and 51 QTL in Y/H (Table 3). About one-third of the QTL in Q/H and only five QTL in Y/H showed significant QEI. Most QTL with significant QEI were the QTL for GYPP and KNPP in both of the populations. Only one of the five and one of the four QTL detected for GYPP and KNPP was stable in Q/H, respectively. Moreover, the joint analysis across all environments revealed some loci that were not detected by using the other two QTL mapping procedures, i.e., the QTL for KWID on chr. 2 in Q/H and the QTL for GYPP on chr. 2 in Y/H (Figs. 4, 5). At the same time, there were also some loci detected only in single environment analysis but not found in the other QTL mapping procedures, i.e., the QTL for KVOL on chr. 1 (umc1727–umc1200) detected only in E2 in Q/H and the QTL for KVOL on chr. 5 (bnlg2305–umc2013) detected only in E3 in Y/H. These QTL were often with small effects and low stability across different environments.

Association between QTL for yield components and kernel-related traits

Hypergeometric probability function was used to investigate genetic associations between yield/yield components (KNPP and KWEI) and kernel-related traits (KDEN, KVOL, KLEN, KWID, and KTHI). The associations between the QTL for GYPP and KVOL ($P = 0.02$) and between the QTL for GYPP and KTHI ($P = 0.01$) were significant only in Q/H. The QTL for KNPP did not show significant association with the QTL for KDEN, KVOL, KLEN, KWID, and KTHI in both populations, except KTHI ($P = 0.01$) in Q/H. The QTL for KWEI were co-located with the QTL for KVOL and KWID in many genomic regions ($P < 0.05$) in both populations. The associations between the QTL for KWEI and KDEN in Q/H and the QTL for KWEI and KTHI in Y/H were also significant ($P = 0.04$ and 0.01, respectively). When considering all of the QTL for yield and yield components, significant associations ($P < 0.05$) between yield/yield components and KVOL, KWID, and KTHI were found in both populations, indicating that kernel size and kernel shape traits closely associated with productivity traits. The phenotypic correlation analysis revealed the highly significant correlation between KLEN and GYPP. However, the association between QTL for KLEN and yield components

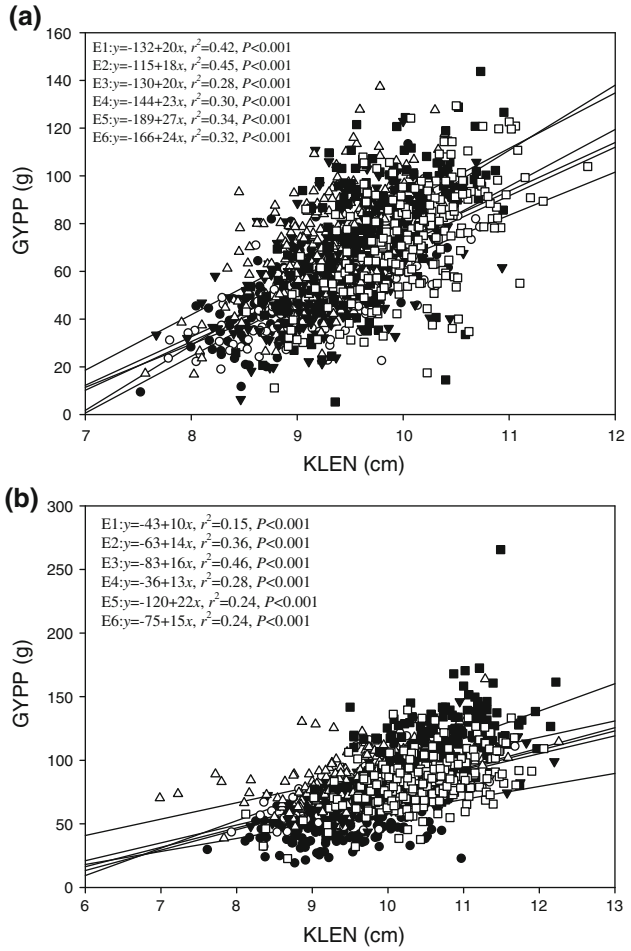

Fig. 2 Relationships between grain yield per plant (GYPP) and 10-kernel length (KELN) of Q/H (**a**) and Y/H (**b**) in six environments. *Different symbols* represent different environments. Different linear regressions for six environments are showed

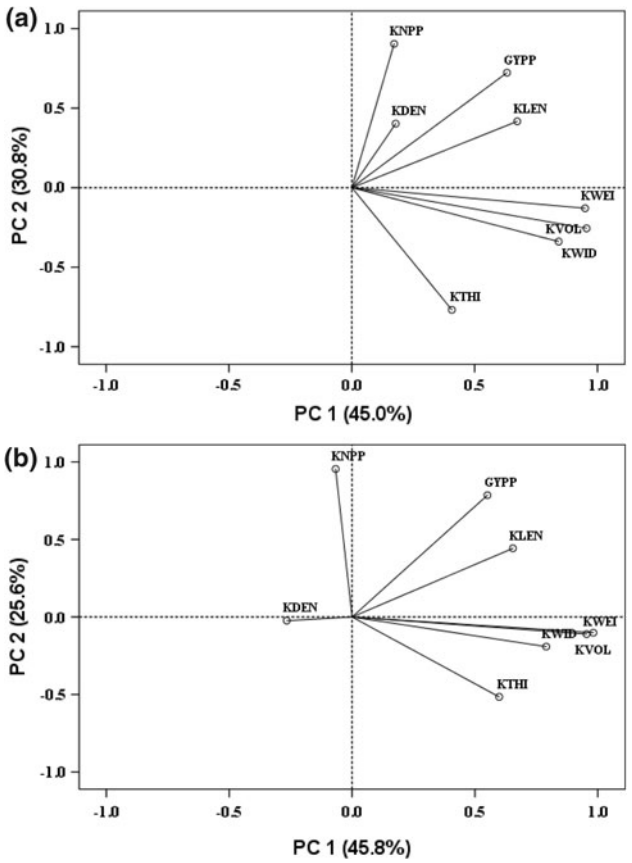

Fig. 3 Bioplot of two major principal components (PC1 and PC2) for the principal components analysis (PCA) (based on correlation matrix) of yield components and kernel-related traits in Q/H (**a**) and Y/H (**b**)

was not significant ($P > 0.05$), with the reason needing further investigation.

In addition, we also found that the QTL for kernel shape traits (KLEN, KWID, and KTHI) significantly associated with the QTL for KWEI and KVOL at $P < 0.05$. Moreover, except for the association between the QTL for KLEN and the QTL for KWID ($P = 0.04$) only in Q/H, there was no common genetic basis between kernel shape traits.

Constitutive QTL

Constitutive QTL refer to the QTL stably detected under different environments. In the present study, the QTL detected in at least four environments with no significant QEI based on the joint analysis across all environments were declared as "constitutive QTL". In total, three and four constitutive QTL in Q/H and Y/H were identified, respectively, of which two were for each of KLEN and KWID, and one was for each of KNPP, KDEN, and KTHI. All seven constitutive QTL accounted for larger proportion of phenotypic variance, and the increasing alleles originated from the same parent in different environments. In addition, the QTL with $R^2 > 10\%$ in at least one environment (based on single environment QTL analysis), furthermore detected based on the joint analysis across all environments were declared as "major" QTL in this study. Only major QTL or QTL detected in at least two environments were considered when looking for concomitant effects for constitutive QTL. All of these QTL were detected concomitant effects on other traits (Figs. 4, 5), suggesting a common genetic basis of these traits through close linkage or pleiotropy.

In Q/H, the first constitutive QTL (*Qqkden1*), for KDEN, was identified on chr. 1 in all six environments with additive gene action within the marker interval of umc1298-bnlg1671 accounted for 7.0% of phenotypic variation based on the joint analysis across all environments (Table 4; Fig. 4). The joint QTL analysis per each location also revealed a significant and stable effect at each

Table 3 Number of QTL detected by three different mapping procedures in the two $F_{2:3}$ populations (Q/H and Y/H)

	GYPP	KNPP	KWEI	KDEN	KVOL	KLEN	KWID	KTHI	Total
Single-environment QTL									
Total (6 env.)	11/7	11/2	12/13	14/2	13/15	11/14	12/9	6/10	90/72
E1	2/0	2/1	1/3	1/1	2/3	3/2	2/2	0/1	13/13
E2	3/3	2/1	3/3	4/0	5/2	4/3	2/2	1/2	24/16
E3	2/2	3/0	3/2	2/1	2/2	0/3	2/2	2/1	16/13
E4	0/1	1/0	2/1	2/0	2/3	1/2	2/0	1/2	11/9
E5	2/1	1/0	1/2	2/0	0/2	1/3	2/2	1/2	10/12
E6	2/0	2/0	2/2	3/0	2/3	2/1	2/1	1/2	16/9
Joint QTL per location									
Total (3 locations)	10(10)/6(5)	8(8)/5(5)	9(8)/13(13)	13(12)/4(4)	17(16)/17(17)	9(9)/15(15)	13(13)/8(8)	10(10)/8(7)	89(86)/76(74)
J1	5(5)/2(2)	3(3)/2(2)	3(3)/3(3)	7(7)/2(2)	6(6)/5(5)	4(4)/4(4)	5(5)/3(3)	2(2)/2(2)	35(35)/23(23)
J2	2(2)/3(3)	3(3)/2(2)	4(4)/5(5)	2(2)/1(1)	5(5)/8(8)	2(2)/4(4)	4(4)/2(2)	5(5)/3(3)	27(27)/28(28)
J3	3(3)/1(0)	2(2)/1(1)	2(1)/5(5)	4(3)/1(1)	6(5)/4(4)	3(3)/7(7)	4(4)/3(3)	3(3)/3(2)	27(24)/25(23)
Joint QTL all environments									
Total	5(1)/6(4)	4(1)/4(3)	11(9)/7(6)	6(3)/4(4)	10(9)/7(6)	6(4)/9(9)	9(8)/8(8)	7(6)/6(6)	58(41)/51(46)

J1, J2, J3 indicate combining data of 2 years for Henan, Beijing, Xinjiang

The numbers in front of "/" indicate QTL detected in Q/H, and the numbers behind of "/" indicate QTL detected in Y/H, the values in parentheses show the number of stable QTL with non-significant QTL × environment interaction

of all three locations (Fig. 4b). Both *Qqgypp1* and *Qqklen1* with positive direction over-dominant gene action had concomitant effects on *Qqkden1* in two environments (E1 and E2) (Table 4; Fig. 4). *Qqgypp1* was also significant and stable across 2 years in Henan based on the joint analysis per location, but both *Qqgypp1* and *Qqklen1* showed significant QEI across all environments (Fig. 4). All of them explained a large part of variation in single environment analysis, for instance 7.2–8.5% for *Qqklen1*, 6.1–13.0% for *Qqkden1*, and 7.6–9.9% for *Qqgypp1* (suppl. Table 2). Qi319 consistently provided the effect-increasing allele for all QTL at this locus.

The second constitutive QTL were identified for KNPP (*Qqknpp6*) on chr. 6 (flanked by umc1656 and umc1796) in four environments accounting for 8.0% of phenotypic variation (Table 4; Fig. 4). Additive gene action was observed for *Qqknpp6*. This genomic region also included a QTL for GYPP (*Qqgypp6*) detected in three environments. QTL for both of the traits were found to have significant and stable effects in Henan and Beijing (Fig. 4b). *Qqgypp6* showed positive direction over-dominant gene action and significant QEI across all six environments. Huangzaosi contributed the favorable alleles at QTL for the two traits (Table 4; Fig. 4).

Qqkwid10 (flanked by bnlg1677 and umc2172) was the third constitutive QTL on chr. 10, accounting for up to 15.9% of phenotypic variation (Table 4; Fig. 4). Meanwhile, QTL for KLEN (*Qqklen10*), KVOL (*Qqkvol10*), and KWEI (*Qqkwei10-2*) detected in 2, 3, and 3 environments, respectively, were observed in the same region. It was worthnoting that *Qqkvol10* could explain 14.4% of phenotypic variation. All the QTL mentioned above, with negative direction partial-dominance, did not show significant QEI across all environments (Table 4; Fig. 4c), and Qi319 consistently provided the favorable allele at the loci for all four traits. The joint QTL analysis per location also revealed significant and stable effects for KLEN and KWID in each of all three locations, for KVOL in Beijing and Xinjiang and for KWEI in Beijing (Fig. 4b).

In Y/H, the first constitutive QTL was identified for KTHI (*Yqkthi1*) on chr. 1 (flanked by bnlg1502 and bnlg1671) in all six environments, explaining for 6.3% of phenotypic variation (Table 5; Fig. 5). Additive gene action was observed for *Yqkthi1*. The joint QTL analysis per location also revealed significant and stable effects in each of all three locations (Fig. 5b). *Yqkvol1* (detected in 2 environments) and *Yqkwei1* (detected in 1 environment) were also found in this genomic region (Table 5; Fig. 5). Both of the single environment analysis and the joint analysis per location demonstrated that *Yqkvol1* was observed in Beijing and Xinjiang, while *Yqkwei1* was only found in Xinjiang (Fig. 5a, b). *Yqkvol1* and *Yqkwei1* were stable within locations (Fig. 5b), but showed significant QEI across all six environments (Table 5; Fig. 5c). Huangzaosi consistently provided the favorable allele for these three traits at the loci across all environments.

The second constitutive QTL was identified for KLEN (*Yqklen4*) on chr. 4 (flanked by umc1662 and umc1299) in four environments, explaining up to 10.8% of phenotypic variation (Table 5; Fig. 5). The joint QTL analysis per

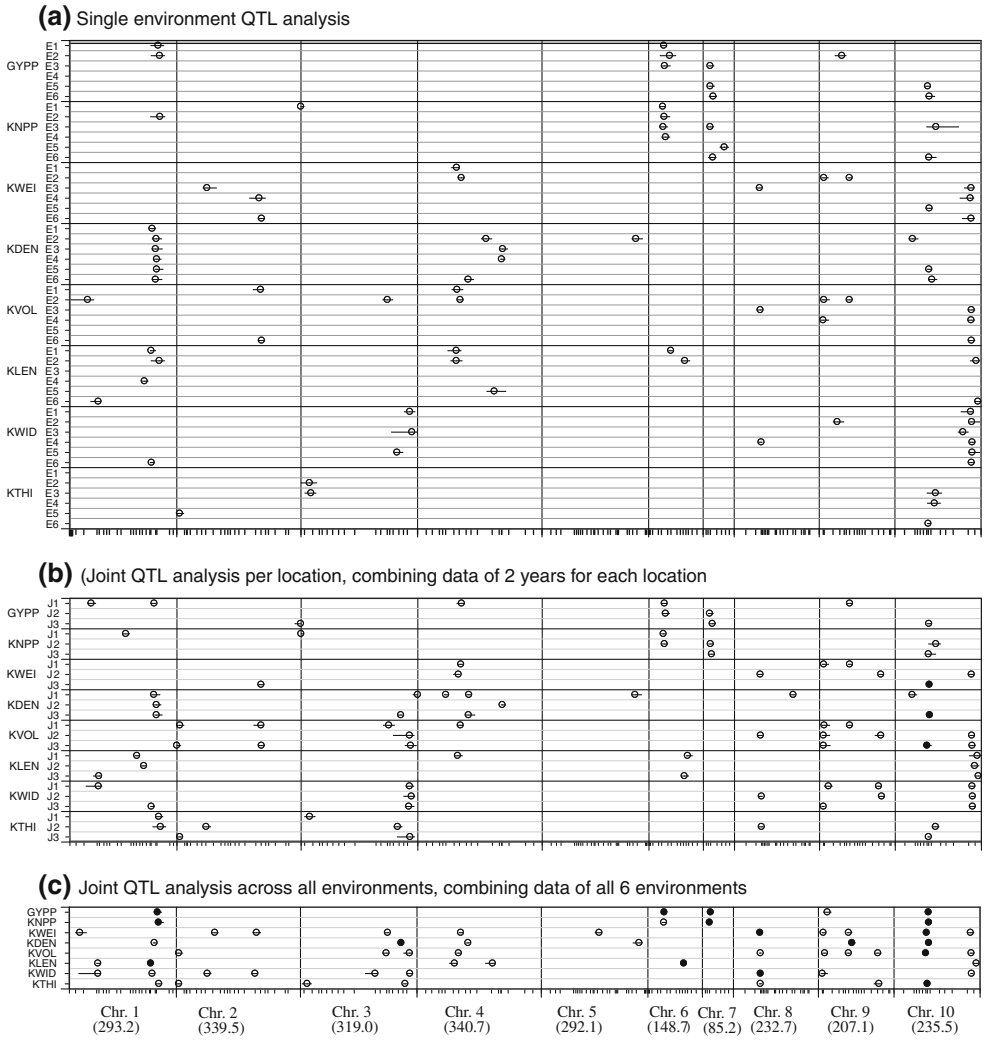

Fig. 4 Genomic regions where the F exceeds the significant threshold for different mapping procedures in Q/H: **a** single environment, **b** per location and **c** across all environments. *Horizontal lines* indicate confidence intervals, and the *plots* indicate the positions of QTL. The *hollow plots* indicate stable QTL with a non-significant QTL × environment interaction while the *solid plots* indicate QTL with significant QTL × environment interaction. *J1*, *J2*, and *J3* indicate combining data of 2 years for Henan, Beijing, and Xinjiang, respectively

location also revealed the significant and stable effect for this QTL in each of all three locations (Fig. 5b). *Yqkvol4*, *Yqkwei4*, and *Yqgypp4* had concomitant effects on *Yqklen4*. Significant and stable effects for *Yqkvol4* and *Yqkwei4* were detected in each of all three locations and even across all environments, and those for *Yqgypp4* in Henan and Beijing were also detected (Fig. 5b, c). Additive gene actions were observed for *Yqklen4*, *Yqkvol4*, and *Yqgypp4*, while partial-dominant gene action was for *Yqkwei4* (Table 5). Higher values for the four traits were consistently conferred by the Huangzaosi alleles.

The third constitutive QTL was identified for KWID (*Yqkwid7*) on chr. 7 (flanked by umc1016 and bnlg1094) in four environments (Table 5; Fig. 5). The joint QTL analysis per location also revealed the significant and stable effect for this QTL in each of all three locations (Fig. 5b). The same genomic region, although influencing KWEI (*Yqkwei7*) in one environment (E1), was still significant and stable across 2 years in Henan and even across all environments. Partial-dominant gene actions were detected for both *Yqkwid7* and *Yqkwei7*. Ye478 contributed to the effect-increasing allele across all environments for the two traits.

The region on the chr. 9 flanking by markers umc1170 and umc1764 was also a constitutive QTL controlling KLEN (*Yqklen9*) which could be detected in four environments (Table 5; Fig. 5). The neighboring region between umc1764 and umc1691 could influence KVOL (*Yqkvol9*) and KWEI (*Yqkwei9*) in 3 and 2 environments, respectively. The allele from Ye478 contributed to the increasing effects for the three traits. The joint QTL analysis per location also revealed significant and stable effects

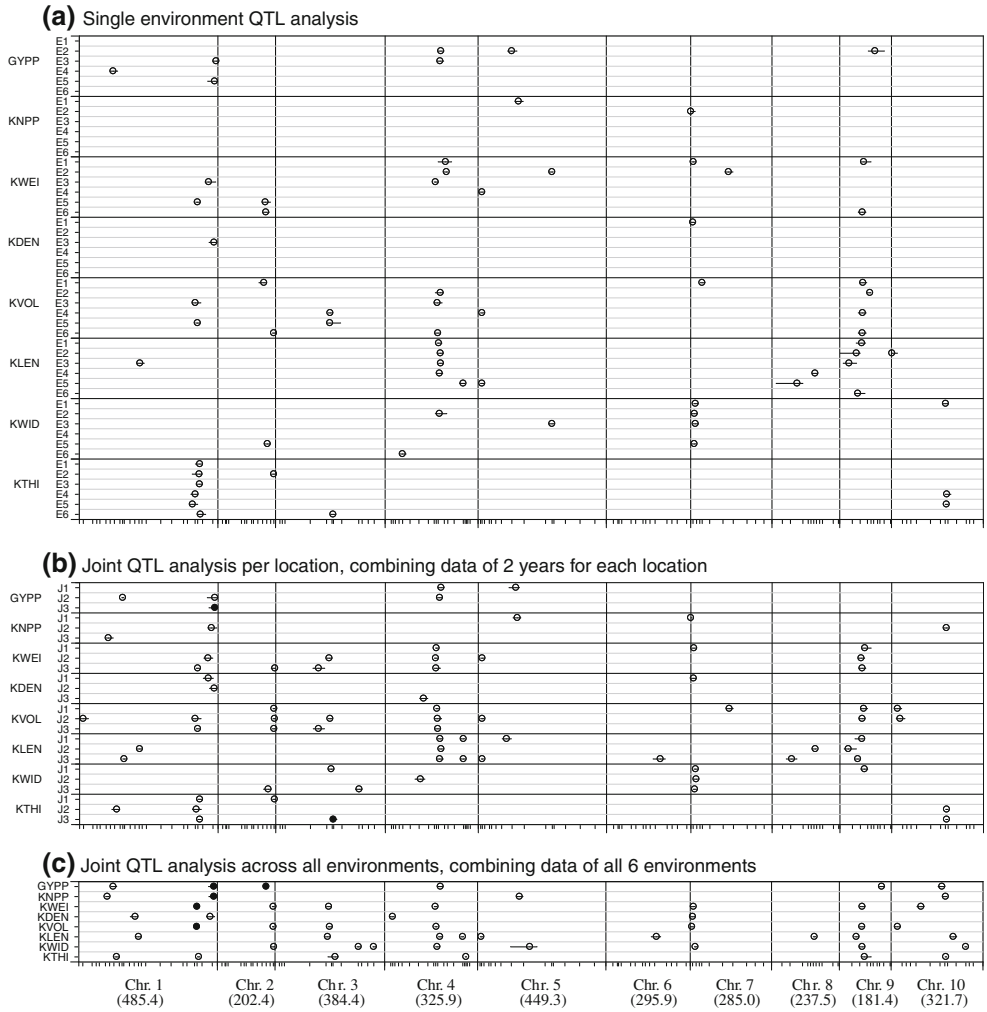

Fig. 5 Genomic regions where the F exceeds the significant threshold for different mapping procedures in Y/H: **a** single environment, **b** per location and **c** across all environments. Notes are the same as those in Fig. 4

for KLEN and KWEI in each of all three locations, and for KVOL in Henan and Beijing (Fig. 5b). All of these three QTL were stable across all environments (Table 5; Fig. 5c).

Epistatic interactions

Six and three pairs of epistatic interactions including additive-by-additive, additive-by-dominant and dominant-by-dominant effects were detected based on single environment QTL analysis for Q/H and Y/H, respectively (data not shown). In Q/H, none of the detected QTL involve in binary epistatic interactions. Three of the six pairs of interactions were detected for KTHI and one for each of KNPP, KWEI, and KDEN. In Y/H, among all three pairs of binary epistatic loci, two were found for KVOL and one for KWEI, and each of the three pairs of interactions involved only one QTL detected. For example, the interaction between marker interval umc1430–umc1170 and *Yqkwei9* on chr. 9 was identified for KWEI in E6. If the alleles at the aforementioned loci from the same parent appeared together, the epistatic interaction between them could decrease KWEI by three times of additive main effect of *Yqkwei9*. In addition, none of the epistatic interactions was consistently detected in different environments.

Discussion

Stability of QTL across environments

The recent genetic improvement of grain yield in maize was mostly attributed to increased stress tolerance of new hybrids, which was achieved by selection for yield stability across target environments (Tollenaar and Lee 2002) and can be carried out by the use of marker-assisted selection (MAS) in the future (Bernardo 2008). However, QEI often

Table 4 Main features of the QTL for yield components and kernel-related traits based on joint analysis across six environments in Q/H

QTL	Flanking marker	Site (cM)	Range	Env.	Effect		R^2 (%)	
					A	D	Q	QE
GYPP								
Qqgypp1	phi308707–umc1009	241.5	236.3–252.5	2	2.83	4.78	2.2	3.6
Qqgypp6	umc1656–umc1796	44.7	39.7–49.7	3	−6.10	6.87	6.9	1.3
Qqgypp7	bnlg1094–bnlg1579	22.8	17.5–28.8	3	3.44	5.08	2.8	7.5
Qqgypp10	phi062–umc1115	94.3	91.3–97.3	2	−1.56	9.44	5.3	3.4
KNPP								
Qqknpp6	umc1656–umc1796	43.7	38.7–48.7	4	−24.62		8.0	
Qqknpp7	bnlg1094–bnlg1579	19.8	16.5–24.8	2	11.48	18.60	2.6	5.9
Qqknpp10	phi062–umc1115	95.3	91.3–98.3	2	−8.57	30.31	4.6	3.5
KWEI								
Qqkwei2	umc1875–umc1080	219.7	209.2–230.4	2	−0.94	0.68	4.5	
Qqkwei4	nc005–umc1869	120.1	115.1–125.1	2	−1.35		7.4	
Qqkwei8	bnlg1352–umc1778	72.7	66.7–76.2	1	−1.16		5.4	0.8
Qqkwei10-1	umc1053–phi062	89.1	86.1–96.3	1	−0.31	0.68	1.3	17.8
Qqkwei10-2	bnlg1677–umc2172	209.7	201.7–218.6	3	1.00	−0.40	4.4	
KDEN								
Qqkden1	umc1298–bnlg1671	233.2	229.2–238.3	6	0.02		7.0	
Qqkden4-1	umc1869–bnlg1784	140.0	135.0–144.0	1	−0.02		6.2	
Qqkden10	phi062–umc1115	95.3	91.3–99.6	2	−0.02	0.01	6.1	7.8
KVOL								
Qqkvol4	nc005–umc1869	114.1	108.3–122.1	2	−0.65		2.8	
Qqkvol9	bnlg1272–umc1170	17.6	9.0–24.6	2	0.81	−0.53	5.2	
Qqkvol10	bnlg1677–umc2172	211.7	207.7–216.7	3	1.42	−0.65	14.4	
KLEN								
Qqklen1	umc1298–bnlg1671	223.2	220.6–230.2	2	0.07	0.11	1.9	1.5
Qqklen4	umc2176–nc005	103.3	89.0–110.3	2	−0.19		6.1	
Qqklen10	umc2172–bnlg1185	225.6	218.6–230.6	2	0.13	−0.10	3.9	
KWID								
Qqkwid3	bnlg1496–umc1010	300.0	291.0–307.0	3	−0.16	−0.09	5.7	
Qqkwid10	bnlg1677–umc2172	212.7	209.7–216.7	6	0.27	−0.11	15.9	
KTHI								
Qqkthi2	umc1419–phi098	7.0	0.0–15.0	1	0.10		3.8	
Qqkthi3	umc2256–bnlg1325	18.7	5.0–29.7	2	0.08		2.9	
Qqkthi10	phi062–umc1115	91.3	90.1–94.3	3	0.09		3.4	13.4

Only QTL with significant effect in at least two environments (QTL confidence interval overlap) and major QTL (QTL with $R^2 > 10\%$ in at least one environment and also detected based on joint analysis across all environments, combining data of all six environments) are reported. QTL characters with bold letters indicate major QTL. The nomination of QTL comprised five parts: the first part "Q" or "Y" stands for QTL detected in Q/H or Y/H, the second part "q" stands for QTL and the third part is the abbreviation of traits. The fourth part, the number stands for chromosome. The fifth part is the serial number of QTL

Site: The distance between QTL and the first marker of the relevant chromosome on the genetic linkage map in centiMorgans

Range, the confidence interval of QTL position

A, additive effect of the QTL; negative values indicate that the alleles for increasing trait value are contributed by common parent Huangzaosi; positive values indicate that the allele for increasing trait value are contributed by another parent Qi319/Ye478

D, dominant effect of the QTL

Env., the number of environments in which the same QTL is detected in the single environment analysis

R^2 (Q), percentage of phenotypic variance explained by the QTL across all environments

R^2 (QE), phenotypic variation explained by main effect QTL × environment interactions across all environments

Table 5 Main features of the QTL for yield components and kernel-related traits based on joint basis across six environments in Y/H

QTL	Flanking marker	Site (cM)	Range	Env.	Effect		R^2 (%)	
					A	D	Q	QE
GYPP								
Yqgypp1-1	bnlg1203–phi109275	121.0	114.0–133.0	1		7.63	4.1	
Yqgypp1-2	umc1009–umc1331	473.8	454.9–478.8	2	−4.43		2.8	9.1
Yqgypp4	umc1662–umc1299	196.0	188.0–203.0	2	−4.47		2.8	
Yqgypp9	phi032–umc2371	148.8	140.8–158.6	1	3.47	5.13	3.6	
KNPP								
Yqknpp5	mmc0481–bnlg1847	147.2	137.2–159.2	1		26.97	4.4	
KWEI								
Yqkwei1	bnlg1502–bnlg1671	414.8	410.8–419.8	1	−0.71	−0.66	3.2	3.1
Yqkwei4	umc1963–bnlg1265	177.9	173.9–183.3	3	−1.04	0.30	5.0	
Yqkwei7	umc1016–bnlg1094	12.0	4.0–17.0	1	1.07	0.38	5.4	
Yqkwei9	umc1764–umc1691	80.6	72.6–89.6	2	0.91	−0.59	4.4	
KDEN								
Yqkden1	bnlg1671–umc1009	460.9	447.9–475.8	1	0.01	−0.01	3.1	
KVOL								
Yqkvol1	umc1082–bnlg1502	413.8	408.8–418.8	2	−0.66	−0.62	3.9	3.0
Yqkvol2	bnlg1045–umc1536	196.5	193.3–198.5	1	−0.80		4.0	
Yqkvol3	phi036–phi374118	191.7	185.7–197.9	2	1.00		6.2	
Yqkvol4	bnlg1265–umc1662	181.3	175.9–184.3	3	−1.05		6.9	
Yqkvol9	umc1764–umc1691	79.6	71.6–87.6	3	0.95	−0.65	6.9	
KLEN								
Yqklen4	umc1662–umc1299	194.0	189.0–200.0	4	−0.31		10.8	
Yqklen5	phi109188–umc1731	12.6	9.6–17.0	1	0.17		3.3	
Yqklen8	umc2175–umc2199	150.6	146.6–154.6	1	−0.24		6.4	
Yqklen9	umc1170–umc1764	59.9	53.9–66.9	4	0.25	−0.10	7.7	
KWID								
Yqkwid7	umc1016–bnlg1094	18.0	15.0–23.7	4	0.24	0.06	9.0	
KTHI								
Yqkthi1	bnlg1502–bnlg1671	420.8	415.8–424.8	6	−0.13		6.3	
Yqkthi3	nc030–umc2166	211.2	185.7–221.2	1	0.06		1.4	
Yqkthi10	umc2016–umc1053	192.3	189.3–195.3	2	0.05	−0.13	4.3	

Notes are the same as those in Table 4

results in change of magnitude of significant QTL effect or direction of additive effects under different environments, and the estimation process of QTL effects also is prone to upwardly bias the effect estimate (Utz et al. 2000; Bohn et al. 2001). Therefore, the application of MAS would be affected.

When the joint analysis per location was used, about 97% of QTL did not show significant QEI in each population, indicating that most QTL across 2 years for each location were stable. However, an increase in proportion of significant QEI was revealed by the joint QTL analysis across all environments, i.e., about 70% of the QTL in Q/H and more than 90% of the QTL in Y/H were detected not to have significant QEI. These findings were supported by Messmer et al. (2009), who indicated that the genetic effects across years at the same location were quite stable for yield components, and secondary traits in tropical maize (80% did not show significant QTL × environment interactions). Ribaut et al. (1997) also found that two QTL for grain yield on chr. 2 and chr. 10, respectively, stably expressed at the same location across 2 years.

Though previous studies reported that the stability of QTL for yield components across different locations, few of which were associated with kernel-related traits. Boer et al. (2007) found in their QTL analysis based on multiple-environment trial data using a mixed-model that QEI effects were important for both grain yield and grain moisture across 12 environments in the U.S. corn belt.

Under non-stress conditions several authors found some yield QTL stably expressed across locations in testcross populations, but when maize inbreds were evaluated for their per se performance in several diverse environments, QEI usually were found to be significant (Stuber et al. 1992; Ajmone-Marsan et al. 1995). When the diverse environments involved different water regimes, the stability of QTL for kernel weight was considerably low, while all of the QTL for grain yield or kernel number were observed to have significant QTL × environment interactions (Messmer et al. 2009; Ribaut et al. 1997). Beavis et al. (1994) suggested that grain yield in maize was controlled by a large number of minor effect QTL sensitive to environments. In the present study, only one QTL were found to be constitutively expressed for KNPP in Q/H. Therefore, it is often difficult to detect QTL for GYPP and KNPP stably expressed in different environments, especially in stress environments.

However, it is much easier to detect QTL for other kernel traits stably expressed across environments (different locations). Several reports revealed that QTL for yield-related traits, such as flowering-related traits and plant structure traits, were more stable than those for grain yield. For example, Vargas et al. (2006) reported that the QTL for grain yield were less stable than those for anthesis-silking interval (ASI). Lima et al. (2006) found that about 75, 50, and 44.4% of the QTL for grain yield, plant height and ear height showed significant QTL × environment interactions across environments (different locations and cropping seasons) in a tropical maize population, respectively. The present study indicated that comparing with the QTL for GYPP and KNPP, the QTL for KWEI, KDEN, KVOL, KLEN, KWID, and KTHI were more stable across different environments.

Comparison with known QTL and QTL consistency in two populations

Seven constitutive QTL were identified on chr. 1, chr. 4, chr. 6, chr. 7, chr. 9, and chr. 10 in the two populations, of which two were for each of KLEN and KWID, and one was for each of KNPP, KDEN, and KTHI. The position of major QTL or QTL detected in at least two environments identified by the joint analysis across all environments were aligned on IBM2008 neighbors map by the common markers. The QTL with overlapping marker intervals for the same traits were considered as "common" QTL between the two populations. Then three genomic regions contain common QTL across the two populations (Tables 4, 5).

Qqkden1 in bin 1.10 seemed located on the same region as *Yqkden1*. *Yqkthi1*, a background specific constitutive QTL, was also detected in bin 1.10. Overall, the two constitutive QTL for KDEN and KTHI were clustered with the QTL for other traits in this genomic region, strongly suggesting the presence of kernel trait-related genes. Several authors reported QTL for starch yield (Lübberstedt et al. 1997), kernel weight, and grain yield (Melchinger et al. 1998; Schön et al. 1994; Ribaut et al. 1997) in this genomic region. *Exg1* conferring cell wall expansion (Kim et al. 2000) is perhaps one of candidate genes for the QTL, but the phenotype of *exg1* is still unclear, which needs further investigation.

Qqknpp6 located in bin 6.02–6.04 specifically expressed in Q/H also influenced *Qqgypp6*. Ajmone-Marsan et al. (1995) identified a major QTL for grain yield on chr. 6 (bin 6.02) in the interval umc059–umc021 accounting for 24.5% of the phenotypic variation under two environments in Italy. The marker interval umc1656–umc1796 is narrower than umc059–umc021 on the IBM2008 neighbors map. QTL for grain yield and kernel weight were also identified to be located in bin 6.02–6.04 by other authors (Ribaut et al. 1997; Melchinger et al. 1998; Kozumplik et al. 1996). It could be concluded that this genomic region seems to be very important for grain yield across different genetic background because of the existence of yield-related major genes.

Qqkwid10 located in bin 10.07 was clustered with *Qqkwei10-2*, *Qqkvol10* and *Qqklen10*. In the Lo964/Lo1016 mapping population, a QTL for grain yield was also found in bin 10.07 (Tuberosa et al. 2002). This region may be specific in some genetic background.

Yqklen4 located in bin 4.05 seems to be very important for the genetic control of grain yield and kernel traits. Not only *Yqklen4* but also some concomitant QTL, i.e., *Qqklen4 and Yqklen4* conferring KLEN, *Qqkvol4 and Yqkvol4* conferring KVOL, and *Qqkwei4 and Yqkwei4* conferring KWEI, were consistently located in the same region in the two populations. Huangzaosi conferred the favorable alleles at the loci across all environments and different genetic background. Ajmone-Marsan et al. (1995) identified a QTL for grain yield on chr. 4 within the interval of umc19–umc42a in two testcross populations. This QTL explained 8.6% of the phenotypic variation for grain yield in the combined analysis across both testers. It was approximately 20 cM apart from our QTL clusters for KLEN, KVOL, and KWEI found in the present study based on the IBM2008 neighbors map.

Yqkwid7 located in bin 7.02 affected not only KWID but also KWEI. Both of these QTL were specific to Y/H. Goldman et al. (1993) indentified a QTL in bin 7.02 for 300-kernel weight in the Illinois long-term selection maize strain. No other report was found in this region.

Yqklen9 with the nearest marker umc1764 in bin 9.02 was clustered with *Yqkwei9* and *Yqkvol9*. Moreover, *Yqkvol9* co-located with *Qqkvol9* in Q/H. Several authors have reported QTL for grain yield (Beavis et al. 1994) and

kernel weight (Goldman et al. 1994; Austin and Lee 1998) in this region. Thus, this region is worth investigating further because of the importance of genetic control of grain yield and kernel weight in maize.

Epistasis

Ma et al. (2007) found that both main effects and epistatic effects were important genetic basis of grain yield and its components in maize, especially epistasis influenced the efficiency of marker-assisted breeding (Li et al. 2003). In this study, only a few epistatic interactions were detected based on single environment analysis. None of the epistatic interactions was consistently detected under different environments. Most of them were the interactions between loci with non-significant main-effect, confirming the results obtained by Ma et al. (2007). The main-effect QTL (M-QTL) could not explain most of the phenotypic variation, while the epistatic effect QTL (E-QTL) also did not explain the entire difference between phenotypic variation explained by M-QTL and the heritability of different traits, as found by Messmer et al. (2009). One of the possibilities was some minor-effect QTL undetected due to the limited population size used in this study (Utz et al. 2000). Another reason was that epistatic interactions were too complicated to be understood thoroughly (Holland 2007; Carlborg and Haley 2004). In maize, a nested association mapping (NAM) populations, consisting of 5,000 recombinant inbred lines (RILs) from 25 families, with 200 RILs per family was constructed, which provided very high resolution and power to detect associations including epistatic interactions (Rafalski 2010). For the trait of flowering time, the lack of detectable epistasis in this NAM population was found (Buckler et al. 2009; McMullen et al. 2009). The epistatic analysis would be more powerful if good quality data from 500 or more F_2 individuals are available, and even larger populations are needed to further explore the contribution of higher-order epistasis (Carlborg and Haley 2004). Therefore, more detailed whole-genome scanning, more powerful bioinformatics tools and larger size of mapping populations are required to illustrate genetic basis of quantitative traits such as grain yield and its components.

Implications for MAS

Because maize is cultivated in diverse agro-ecological conditions, genotype × environment interaction is of great concern in maize breeding. High and stable yield are the most important target in maize breeding. However, grain yield is the final outcome of inter-related polygene-controlled developmental processes and is sensitive to environments (Peleg et al. 2009). The complexity of the genetic control of grain yield increases the difficulties of direct selection. Many secondary morpho-physiological traits were used to improve grain yield indirectly. Kernel traits, especially kernel shape traits, with high heritability and high correlation to yield could be considered, as demonstrated in this study not only by the phenotypic analysis but also by the analysis of hypergeometric probability function.

Ribaut et al. (1997) pointed out that when improving yield under drought, MAS using only the QTL involved in the expression of yield components appears not to be the best strategy. The authors put forward that marker-assisted selection for yield should be considered in a combination of QTL with major effect and stable expression across environments for traits significantly correlated with yield. The QTL for GYPP and KNPP detected in the present study were less stable than QTL for KWEI, KDEN, KVOL, KLEN, KWID, and KTHI across different locations. Therefore, the use of marker-assisted selection for kernel shape traits would be easy and reliable to improve grain yield.

QTL with major effect and stable expression across environments and genetic backgrounds are most important for MAS (Ribaut and Hoisington 1998). Three of seven constitutive QTL were detected to be consistently expressed across the two populations. They perhaps have higher values in MAS for improving yield in maize. However, more validation is needed if MAS will be extended to other breeding populations with different genetic background. Moreover, xenia effect should be considered in the process of further QTL validation.

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