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ORIGINAL PAPER

Genetic analysis and major QTL detection for maize kernel size and weight in multi-environments

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Abstract

Key Message **Twelve major QTL in five optimal clusters and several epistatic QTL are identified for maize kernel size and weight, some with pleiotropic will be promising for fine-mapping and yield improvement.**

Abstract Kernel size and weight are important target traits in maize (*Zea mays* L.) breeding programs. Here, we report a set of quantitative trait loci (QTL) scattered through the genome and significantly controlled the performance of four kernel traits including length, width, thickness and weight. From the cross V671 (large kernel) × Mc (small kernel), 270 derived $F_{2:3}$ families were used to identify QTL of maize kernel-size traits and kernel weight in five environments, using composite interval mapping (CIM) for single-environment analysis along with mixed linear model-based CIM for joint analysis. These two mapping strategies identified 55 and 28 QTL, respectively. Among them, 6 of 23 coincident were detected as interacting with environment. Single-environment analysis showed that 8 genetic regions on chromosomes 1, 2, 4, 5 and 9 clustered more than 60 % of the identified QTL. Twelve stable major QTLs accounting for over 10 % of phenotypic variation were included in five optimal clusters on the genetic region of bins 1.02–1.03, 1.04–1.06, 2.05–2.07, 4.07–4.08 and 9.03–9.04; the addition and partial dominance effects of significant QTL play an important role in controlling the development of maize kernel. These putative QTL may have great promising for further fine-mapping with more markers, and genetic improvement of maize kernel size and weight through marker-assisted breeding.

Abbreviations

KL	20-Kernel length
KW	20-Kernel width
KT	20-Kernel thickness
HKW	100-Kernel weight
CIM	Composite interval mapping
MCIM	Mixed linear model-based composite interval mapping
QTL	Quantitative trait loci
SSR	Single sequence repeat
MAS	Marker-assisted selection
QEI	QTL × environment interaction

Introduction

Maize (*Zea mays* L.) is one of the most important cereal crops—it is widely consumed and plays a crucial role in sustaining food security. In addition, forage production and industrial energy require maize as a raw material. The wide range of demand makes grain yield a major target of maize breeding. Grain yield is a quantitative feature with a complex genetic basis and various regulatory quantitative trait loci (QTL)/genes affected by environmental factors (Austin and Lee 1996; Beavis et al. 1994; Messmer et al. 2009). Compared with grain yield, yield components have higher heritability and better stability across environments (Messmer et al. 2009; Peng et al. 2011). Dissecting a complex quantitative trait into several related components

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will be aided and so increase their genetic effect by identifying more QTL associated with such causal traits (Yang et al. 2012) as yield components, physiological processes during grain filling and kernel internal components (Austin and Lee 1996, 1998; Goldman et al. 1993; Gupta et al. 2006; Li et al. 2007, 2009, 2012, 2013; Liu et al. 2011; Lu et al. 2011; Messmer et al. 2009; Veldboom and Lee 1996; Wassom et al. 2008). During domestication, kernel size and weight are recognized as important yield components for improving grain yield (Doebley et al. 2006). Kernel size, referring to the space bounded by the husks and measured by kernel length, width and thickness, serves as a component of grain yield that determines kernel weight (Borrás and Otegui 2001; Li et al. 2009; Xing and Zhang 2010). Grain yield has been demonstrated to significantly correlate with kernel size, especially kernel length (Li et al. 2009, 2013). Meanwhile kernel size is also a positive factor influencing the end-use quality of maize (Gupta et al. 2006), grain filling (Liu et al. 2011) as well as seedling vigor in early growing maize in cool humid regions (Revilla et al. 1999). Therefore, improving kernel size and weight is a prime breeding target to facilitate the improvement of maize yield.

Great progress has been made in identifying major QTL and isolating underlying genes for kernel size and weight in grain crops, such as rice (Ishimaru 2003; Li et al. 2011; Qiu et al. 2012; Song et al. 2007; Wan et al. 2006, 2008), soybean (Han et al. 2012; Xu et al. 2011), wheat (Breseghello and Sorrells 2007; Ramya et al. 2010; Sun et al. 2009) and barley (Ayoub et al. 2002; Backes et al. 1995). Especially for rice, several genes, *GS3* (Fan et al. 2006), *qGL3* (Zhang et al. 2012) and *GW2* (Song et al. 2007), *GS5* (Li et al. 2011), *GW8* (Wang et al. 2012b) and *qSW5*/*GW5* (Shomura et al. 2008; Wan et al. 2008), which are associated with seed size and grain yield have been identified and cloned through map-based cloning. Results of previous studies revealed that grain yield is significantly determined by kernel-size traits.

Compared with related research in rice, the molecular cloning of genes associated with kernel size and weight has lagged behind in maize (Arumuganathan and Earle 1991). Kernel size and weight, as important agronomic traits and yield components, can be used to facilitate maize yield and have been increasingly attractive in molecular genetics in recent years (Austin and Lee 1996; Gupta et al. 2006; Li et al. 2009, 2012; Peng et al. 2011; Ribaut et al. 1997). Mutant analysis was used to demonstrate the first gene *gln1-4* (glutamine synthetase) known to influence maize kernel size (Martin et al. 2006); in addition, *ZmGS3* and *ZmGW2* in maize, consistent with previous relevant QTL analyses, were identified to be highly homologous with rice *GS3* and *GW2* through an orthologous cloning method (Li et al. 2010a, b). However, the effects on kernel size of these two maize genes were not as remarkable as that of their orthologs in rice (Li et al. 2010a, b). Therefore, more attention should be paid to 'mining' favorable QTL/genes to enhance the understanding of the genetic basis of maize kernel-related traits, and applying them to marker-assisted selection (MAS).

The lack of consistent QTL across environments is usually the major impediment to applying the achievements generated from a handful of studies on QTL mapping and genetic analysis for maize yield, particularly for kernel-related traits. Recently, Peng et al. (2011) reported that QTL for kernel-related traits were clearly more stable than that for grain yield across diverse environments, indicating that more efficient selection would be performed if robust QTL for kernel-related traits were fine mapped.

In the present study, an $F_{2:3}$ segregating population derived from Mc × V671 was used to (a) identify QTL for kernel size and weight in multiple agri-ecological environments; (b) detect the QTL × environment interactions (QEIs) to find crucial stable QTL and characterize the epistatic QTL for kernel-related traits; and (c) investigate the genetic basis and correlation between kernel size and weight. This study aims to improve the understanding of the intricate genetic basis of kernel size and weight and to contribute favorable kernel-related QTL for fine-mapping to aid yield improvement in maize breeding.

Materials and methods

Plant materials

An F_2 population derived from a cross between two maize elite inbred lines, Mc and V671, which have significantly different kernel size and were created for QTL analysis. Mc has small kernels while V671 has much larger kernels (Fig. 1). The F_2 population was planted in Hainan, China during the winter of 2010; and 270 F_2 plants were successfully self-pollinated. The seeds of the 270 $F_{2:3}$ families with less missing phenotypic data according to the subsequent phenotypic analysis were harvested from the 270 F_2 selfed-plants, respectively, and used for validating the phenotype in multi-environments.

Field trials

The trials were performed at three experimental stations located in Wuhan (WH), Huanggang (HG) and Enshi (ES), during 2011 and 2012, respectively. Each location and year combination was considered as an experimental environment. Abbreviations were used to identify the different environments, i.e. WH11, HG11, ES11, HG12 and ES12 indicated environments of Wuhan in 2011, Huanggang

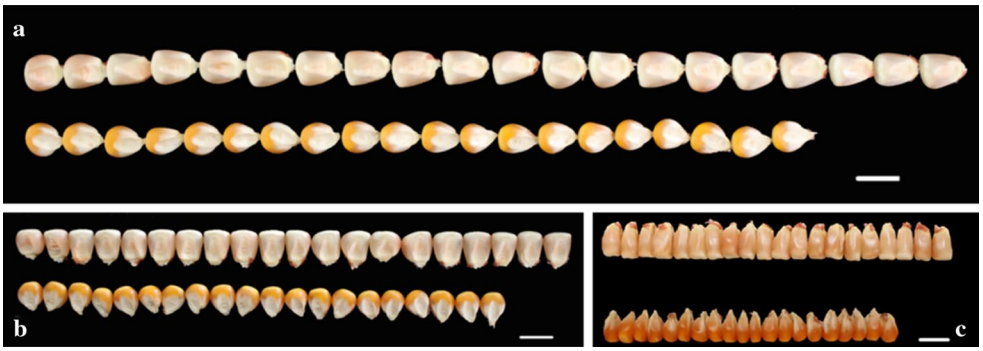

Fig. 1 Kernel phenotypes of the two parental *inbred lines* used for QTL mapping in this study. **a** 20-kernel length, **b** 20-kernel width, **c** 20-kernel thickness. *Scale bars* 10 mm for **a**, **b** and **c**. Kernels in *upper lines* belong to the large-kernel parent V671 and those in the *lower lines* from the small-kernel parent Mc

in 2011, Enshi in 2011, Huanggang in 2012 and Enshi in 2012, respectively.

All trials were laid out as randomized complete block designs with two replications, except for ES11 which was not replicated. Each plot consisted of two rows with spaced 0.30 m apart on a raised bed, 3 m in length, 0.50 m in width and with spacing between plots of 0.25 m. Each genotype was grown in a single-row. Twelve open-pollinated individual plants were harvested, and all shelled from the middle part of ears at maturity, for each genotype in the five trial circumstances, respectively. Then kernels were bulked for each genotype and used to measure the kernel-related traits. Kernel-related traits were measured for each genotype as follows:

100-kernel weight (HKW, g) was the average weight of three repeated measurements of 100 kernels randomly sampled from the bulked kernels and weighed by electronic balance; Kernel length (KL, mm), width (KW, mm) and thickness (KT, mm) were estimated by the average of three replicated measurements of 20 kernels randomly chosen from the bulked kernels using electronic digital calipers.

Phenotypic data analysis

The phenotype performance of kernel-related traits in single environment was determined by the average of each family from two replications. SPSS17.0 software (http://www.spss.com) was used to calculate the variance components including genotype, environment, replication and interaction between genotype and environment of each trait by general linear model (GLM) program. Broad-sense heritability (H^2) for each trait was estimated as described by Hallauer and Miranda (1998).

$$H^2 = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_{ge}^2/n + \sigma_\varepsilon^2/rn}$$

Here, σ_g^2 is the genetic variance, σ_{ge}^2 is the interaction of genotype with environments, σ_ε^2 is the residual error, while n is the number of environments with replications, and r is the number of replications per environment. σ_g^2, σ_{ge}^2 and σ_ε^2 were obtained from variance components of GLM analysis by SPSS17.0 software as well as by regression analysis.

Phenotypic correlation coefficients (r) between kernel-related traits in each environment were estimated by SPSS17.0 software (http://www.spss.com). Coefficients of genotypic correlations (r_g) between two traits were conducted with PLABSTAT software (Utz 1997).

Genotyping and the construction of genetic linkage map

Total genomic DNA was extracted and purified with modified CTAB method (Saghai-Maroof et al. 1984) from the fresh leaf tissue of 270 individual F_2 plants whose kernel-related traits were estimated based on their $F_{2:3}$ family progeny test. In accordance with bin location among genomes, a total of 1102 single sequence repeat (SSR) molecular markers chosen from the maize genome database (http://www.maizegdb.org/) were used to detect polymorphisms between the two parental lines, using the protocol available at http://www.maizegdb.org/documentation/maizemap/ssr_protocol, with slight modification. The 270 F_2 individuals were eventually genotyped by 256 distinct co-dominant SSR markers. PCR products were separated on 6 % denaturing polyacrylamide gels with a 19:1 ratio of acrylamide:bisacrylamide and then silver stained as described by Santos et al. (1993).

A molecular linkage map (Fig. 2) of total length 1,351.7 cM across maize genome with an average interval between adjacent markers of 5.28 cM, was constructed by Mapmaker/EXP V3.0 software (Lander et al. 1987; Lincoln et al. 1992) with 'error detection on' at logarithm of odds (LOD) threshold >3.72. The Kosambi mapping function (Kosambi 1943) was used to calculate genetic distance. The linear order of most markers in the linkage map was

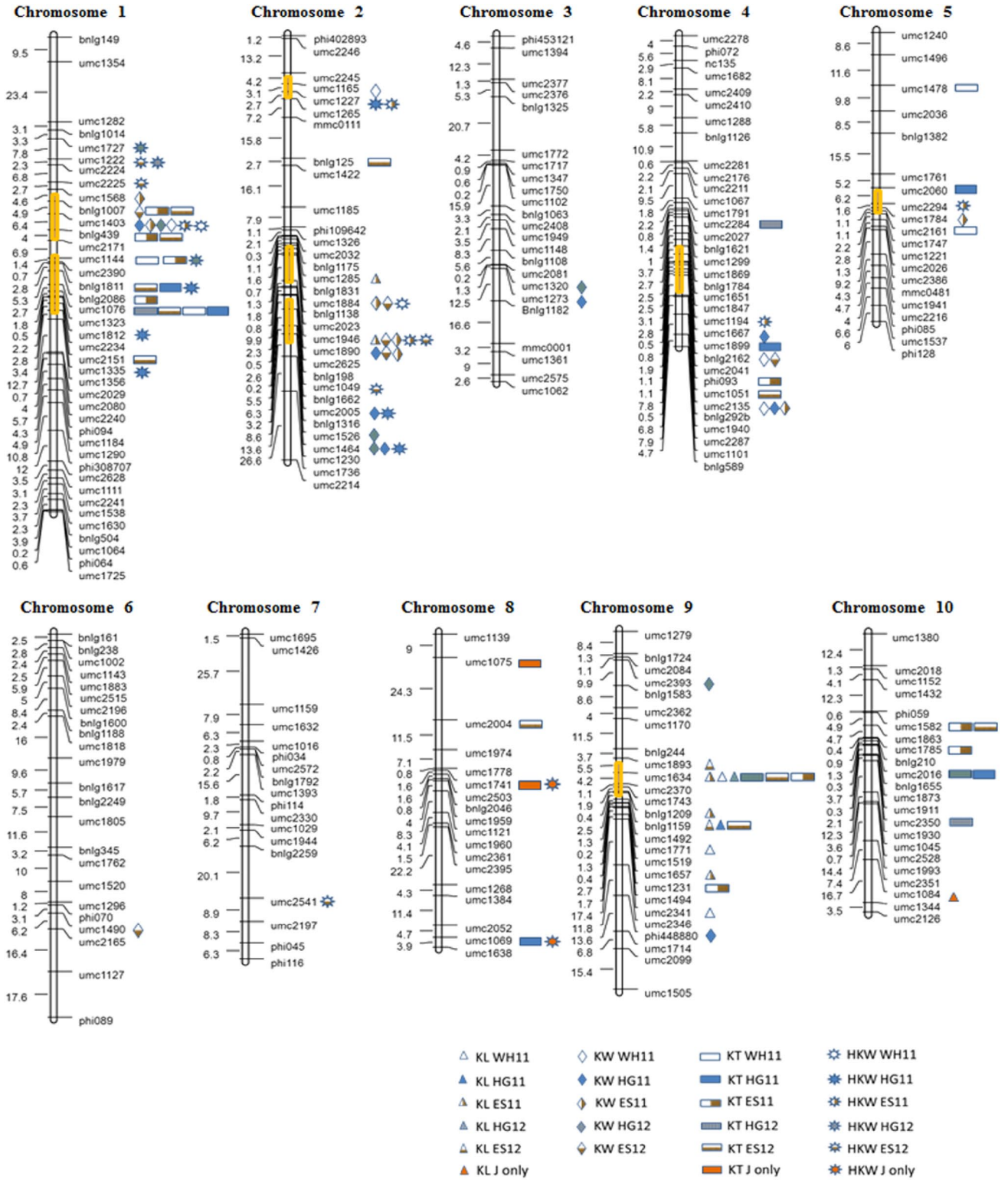

Fig. 2 Distribution of identified quantitative trait loci (QTL) for kernel size and kernel weight on genetic linkage maps in this study. The marks denoted peak positions of QTL. WH11, HG11 and ES11 represent Wuhan, Huanggang and Enshi in 2011, respectively; HG12 and ES12 represent Huanggang and Enshi in 2012, respectively. KL (20-kernel length), KW (20-kernel width) and KT (20-kernel thickness) are measured in the unit of millimeter (mm); and the unit of HKW (100-kernel weight) is gram (g). J only represented the QTLs that were detected through joint mapping only. *Numbers* on the *left* side are the genetic distances between two flanking markers with the unit of centiMorgan (cM). The eight important QTL clusters' regions overlapping with that derived from previous studies were designed as *orange color box* on chromosome bars (color figure online)

in agreement with their order of physical positions (Supplementary Fig. S2).

QTL mapping

QTL analysis was performed by composite interval mapping (CIM), presented in the Windows QTL Cartographer software 2.5 (Wang et al. 2007), at 1 cM walk speed and with 10 cM window size to determine whether the two adjacent test-statistic peaks represented two QTLs. Model 6 in CIM was employed to identify QTL for each trait in each environment, with the values greater than LOD threshold considering 1,000 permutations ($P = 0.05$) to determine whether the presence of a QTL at a certain chromosomal region was significantly associated with target trait, as suggested by Lander and Kruglyak (1995). QTLs which were stably identified from different environments for a target trait with clearly similar positions (overlapping 1-LOD confidence intervals provided by software) were assumed to be the same. QTL, which could be identified in multiple environments and explain more than 10 % of phenotypic variation, was considered as major QTL. QTL detected for different traits with overlapped confidence intervals and common marker(s), or couples of overlapped QTL with distance less than 2 cM was defined as a QTL cluster in which at least one stable major/large effect (phenotypic variation explained >10 %) QTL was included. The phenotypic data used for QTL analysis of each trait was based on the means of two replications in a single environment.

Analysis of joint QTL, binary epistatic interaction in a single environment and QEIs based on the datasets of all experimental environments was performed by mixed linear model-based composite interval mapping (MCIM) with best linear unbiased predictors (BLUP) for random effect prediction of QTLNetwork software version 2.0 (Yang et al. 2007). Window size, working speed and filtration window were set at 10, 2 and 10 cM, respectively. The *F*-test using Henderson method III was employed to determine significance, and the critical *F*-value was estimated by 1,000 permutation tests (Doerge and Churchill 1996). QTL designations were defined adopting the nomenclature of McCouch et al. (1997). The designation for a QTL starts with 'q', followed by an abbreviation of the trait name, then the number of the chromosome on which the QTL was located, and finally, the serial number assigned to the related trait of QTL on a specific chromosome. The last number was omitted in QTL nomenclature under the situation that there is only one QTL detected on the specific chromosome for a trait. In addition, if the QTL was identified only by joint analysis among all environments but not a single-environment QTL detection, then 'J' was placed after the numbers representing the chromosome of the significant QTL.

Results

Trait performance

The two parents, Mc and V671, showed highly significant differences ($P < 0.001$) in all examined kernel-related traits (Fig. 1; Table 1) with higher values generally for V671. Among the $F_{2:3}$ families, most traits were approximately normally distributed, and there were wide variations in the performance measurements at the three locations during the 2 years (Table 1 and Supplementary Fig. S1)—notably, the phenotypic values of all four traits exhibited obvious bi-directional transgressive segregation in all environments, indicating polygenic quantitative genetic control. Broad-sense heritability (H^2) of the four kernel-related traits ranged from 0.881 (KL) to 0.944 (KW), suggesting that genetic factors played an important role in the formation of these traits. The highly significant difference ($P < 0.001$) was found in genotype and environments for all traits, and the interactions G × E were significant for KW, KT and HKW. The variances of the replications for all traits were non-significant ($P < 0.05$) except for KW (Table 2), which is the reason that the mean of two replications in one location for each genotype was used for the subsequent QTL mapping.

The phenotypic and genotypic correlation coefficients between kernel-related traits across environments revealed highly significance in $F_{2:3}$ families (Table 3). Only in HG11 and HG12 were there no significant phenotypic correlations between KL and KW, with significant ($P < 0.01$) positive phenotypic and genotypic correlations in the other three environments and among all environments, respectively, suggesting that differences between experimental locations affected kernel development. It is noteworthy that a significant negative phenotypic and genotypic correlation only occurred between KT and KL across all environments ($P < 0.01$). Outstanding phenotypic and genotypic correlations were found between KL and KW, KW and KT, and between HKW and these kernel-size traits, implying the important role of kernel size in determining HKW and potential for simultaneous improvement. Interestingly, similar results were reported in previous study using $F_{2:3}$ families (Li et al. 2009; Peng et al. 2011). Simultaneously, regression analysis revealed that, the largest contributor to HKW was KT, with KL and KW followed (Supplementary Table S1). Overall, the significant correlation of the majority of character pairs indicated closely genetic association among kernel size and weight, and that the population was deserved for further studies of QTL mapping for these kernel-related traits.

Table 1 Phenotypic performance of the four maize kernel-related traits in $F_{2:3}$ families under five environments

Trait	Env.	Mc	V671	$F_{2:3}$ families							
				Mean	SD	Min	Max	% TS	Skew	Kurt	*P* value
KL	WH11	186.2	205.1	193.8	12.3	160.6	224.8	43.6	−0.05	−0.08	0.76
	HG11	189.9	201.4	214.6	11.6	187.5	249.9	87.4	0.27	0.02	0.18
	ES11	205.1	220.6	237.9	12.9	195.9	281.4	91.7	−0.11	0.32	0.51
	HG12	180.4	208.0	208.1	13.7	163	249.6	57.7	−0.42	0.67	0.01
	ES12	200.3	217.8	224.1	13.1	182.8	270.8	72.3	0.07	0.83	0.23
KW	WH11	150.5	169.0	154.0	8.0	134.5	180.4	36.5	0.14	−0.02	0.74
	HG11	152.1	168.8	159.3	8.0	132.7	178.3	32.0	−0.07	−0.12	0.52
	ES11	156.9	168.5	163.0	9.2	139.4	193.9	52.7	−0.02	0.15	0.53
	HG12	156.6	173.8	165.4	8.5	133.1	189.5	31.3	−0.21	0.37	0.39
	ES12	164.5	175.2	163.9	8.4	143.6	185.8	60.6	0.05	−0.36	0.37
KT	WH11	83.2	104.9	98.8	9.4	77.1	123.5	26.3	0.18	−0.28	0.13
	HG11	92.5	117.8	99.1	7.6	73.2	119.7	18.9	0.01	0.02	0.90
	ES11	84.2	95.6	86.7	6.0	71.5	105	42.0	0.13	−0.01	0.79
	HG12	90.5	103.7	92.4	7.2	75.5	118	46.9	0.29	0.26	0.27
	ES12	83.9	86.7	85.9	5.4	72.5	100.9	78.8	0.14	−0.20	0.38
HKW	WH11	22.5	26.3	22.5	2.1	17.4	29.4	56.0	0.14	0.03	0.52
	HG11	22.1	26.6	24.6	2.3	19.3	31.7	32.6	0.12	−0.22	0.65
	ES11	23.4	28.3	28.8	3.4	20.3	37.7	64.8	−0.04	−0.37	0.24
	HG12	20.0	26.7	24.6	2.3	17.3	30.1	24.4	−0.41	0.02	0.02
	ES12	22.9	28.4	25.1	3.7	12.9	33.6	43.8	−0.42	0.23	0.03

V671: parent inbred line with large kernel; Mc: parent inbred line with small kernel

KL (20-kernel length), KW (20-kernel width) and KT (20-kernel thickness) are measured in the unit of millimeter (mm); and the unit of HKW (100-kernel weight) is gram (g)

Env., represents environment; WH11, HG11 and ES11 represent Wuhan, Huanggang and Enshi in 2011, respectively; HG12 and ES12 represent Huanggang and Enshi in 2012, respectively

%TS, represents the transgressive segregation which refers to the percentage of $F_{2:3}$ families with phenotype beyond the range of two parents

P value, results from the Shapiro–Wilk test for normalized detection

SD standard deviation

QTL analysis

The results of QTL analysis for the four kernel-related traits in $F_{2:3}$ families are shown in Fig. 2 and the analyses of putative QTL are summarized in Table 4 and Supplementary Table S2. A total of fifty-five QTLs were identified for four traits through single-environment QTL analysis and spread over all ten chromosomes (Table 4; Fig. 2). The phenotypic variation explained by individual QTL ranged from 0.46 (*qHKW7*) to 20.56 % (*qKW1-2*). Over 49.09 % of the identified QTL had positive additive effect, indicating that alleles from the large-kernel parent V671 contributed on increasing phenotype. Results concerning the QTL detected in the study are presented below.

20-Kernel length

Six QTLs for KL were identified by single-environment mapping and individually accounted for 1.18–12.92 % of the phenotypic variation while they explained 8.84–40.03 % together of KL variation in each environment (Table 4; Fig. 2). The major QTL *qKL9-1* accounted for 2.39–11.98 % of the phenotypic variation with LOD value 2.77–7.38. Another major QTL, *qKL9-2*, explained up to 12.92 % of phenotypic variation with higher LOD of 3.89–8.27. The positive additive effects of all QTL on chromosome 9 indicated that their alleles were derived from large-kernel parent V671. In contrast, the negative additive effect of the rest two environment-specific QTL on chromosome 2 indicated that alleles from small-kernel parent Mc at these loci were beneficial for increasing KL. The four KL QTLs on chromosome 9 mainly characterized by A or PD effects, while the other two on chromosome 2 showed OD and D effects.

20-Kernel width

KW was governed by 16 QTLs dispersed on chromosomes 1, 2, 3, 4, 5 and 9. Each explained 1.7–20.51 % of

Table 2 Analysis of variance (ANOVA) for kernel-related traits of $F_{2:3}$ families in four environments

Trait	Source of variation	*F*	H^2
KL	Environment (E)	476.675***	0.881
	Genotype (G)	2.971***	
	Replication	2.004	
	G × E	1.111	
KW	Environment (E)	267.217***	0.944
	Genotype (G)	6.921***	
	Replication	6.090*	
	G × E	1.145*	
KT	Environment (E)	393.669***	0.920
	Genotype (G)	4.880***	
	Replication	1.771	
	G × E	1.186**	
HKW	Environment (E)	88.687***	0.884
	Genotype (G)	3.231***	
	Replication	0.576	
	G × E	1.201**	

H^2 the broad-sense heritability

*, ** and *** indicate significant level at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively

Table 3 Phenotypic (*r*) and genotypic (r_g) correlation coefficients between kernel-related traits across five environments

Trait	Env.	KL	KW	KT
KW	WH11	0.23**		
	HG11	0.06^{ns}		
	ES11	0.19**		
	HG12	0.09^{ns}		
	ES12	0.13**		
	r_g	0.17*		
KT	WH11	−0.54**	0.16**	
	HG11	−0.39**	0.40**	
	ES11	−0.17**	0.56**	
	HG12	−0.47**	0.24**	
	ES12	−0.27**	0.54**	
	r_g	−0.43**	0.51**	
HKW	WH11	0.32**	0.63**	0.30**
	HG11	0.26**	0.62**	0.44**
	ES11	0.50**	0.66**	0.56**
	HG12	0.32**	0.58**	0.21**
	ES12	0.44**	0.45**	0.32**
	r_g	0.29**	0.92**	0.57**

r_g, genotypic correlation coefficients of two kernel-related traits among four environments with replications

Env. represents environments; WH11, HG11 and ES11 represent Wuhan, Huanggang and Enshi in 2011, respectively; HG12 and ES12 represent Huanggang and Enshi in 2012, respectively

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

phenotypic variation with *qKW1-2* contributing the highest percentage in most environments. Among them, half QTLs were repeatedly detected in multiple environments. Notably, *qKW1-2* was the only KW QTL found in all five environments with LOD varied among 6.86–12.38. Besides, another three major QTLs (*qKW2-2*, *qKW1-2* and *qKW4-2*) and four environment-specific QTLs were identified in correspondence with joint analysis. Of which, *qKW1-2*, *qKW2-3* and *qKW5* were co-located with QTL for HKW, *qHKW1-4*, *qHKW2-3* and *qHKW5* on chromosomes 1, 2 and 5, respectively. Out of the 16 QTLs associated with KW, the positive additive effects of six QTLs on chromosomes 1, 4 and 9 indicated that their positive alleles (alleles which increased the trait) were consistently contributed by the large-kernel parent V671, while the positive alleles of the other ten QTLs on chromosomes 2, 3, 5 and 6 were contributed by small-kernel parent Mc. All of the QTL associated with KW showed A or PD effects, except for *qKW3*.

20-Kernel thickness

A total of 18 QTLs influencing the KT were identified on chromosomes 1, 2, 4, 5, 8, 9 and 10 in the present study, individually explaining 0.84–17.98 % of phenotypic variation and totally accounting for 39.23–63.6 % of KT variation in each environment. Among them, nine QTLs were significant in multiple environments and the additional nine were environment-specific. The V671 alleles had a positive effect on increasing KT for nine QTLs distributed on chromosomes 1, 4, 5 and 8, including two location-specific major QTL (*qKT1-1* and *qKT1-2*), one major (QTL *qKT1-4)* detected across all five environments with 5.07–17.98 % of the phenotypic variation and another major QTL (*qKT1-3*) explaining 3.09–14.93 % of the phenotypic variation in four environments. Five of the 18 QTLs detected for KT were located on the same map position with the QTL for KW and HKW. Two QTLs, *qKT9-1* and *qKT9-2* on chromosome 9 were co-located with QTL for KL and the major QTL *qKT1-4* on chromosome 1 was corresponding with one major QTL for HKW. Thirteen QTLs for KT showed A or PD effects and the rest QTLs were basically characterized by dominance effects (D or OD).

100-Kernel weight

Fifteen QTLs influencing the HKW were detected (Table 4) with seven on chromosome 1, five on chromosome 2 and one each on chromosomes 4, 5, and 7. Six of the 15 QTLs were identified across 2 environments and nine were environment-specific QTL. All positive alleles of QTL on chromosomes 1 and 4 were derived from large-kernel parent V671. The phenotypic variation explained by

Table 4 Putative QTL for maize kernel size and weight in $F_{2:3}$ families through single-environment QTL mapping

Trait	QTL[a]	Marker interval	Range (cM)	Size (Mb)[b]	Env.[c]	Peak position (cM)	Bin[d]	*A*[e]	*D*[f]	Gene action[g]	PVE[h] (%)	LOD
KL	*qKL2-1**	bnlg1175-umc1285	75.5–79.7	9.60	ES11	78.71	2.04	2.09	2.86	OD	1.18	4.45
	qKL2-2	umc2023-umc1890	82.3–94.2	22.18	ES11	90.91	2.07	2.84	2.8	D	2.28	3.85
	qKL9-1	umc1893-umc1634	48.3–59.5	6.48	ES12	49.61	9.02	−5.17	3	PD	7.67	3.12
					WH11	55.11	9.03	−2.75	−1.96	PD	2.4	2.77
					ES11	56.11	9.03	−6.38	−0.57	A	11.99	7.38
					HG12	56.11	9.03	−5.89	3.96	PD	8.84	3.35
	*qKL9-2**	bnlg1209-umc1771	60.3–65.7	11.46	ES11	61.41	9.04	−6.36	−0.85	A	11.96	8.27
					ES12	61.71	9.04	−5.39	1.71	PD	8.3	3.89
					HG11	62.71	9.04	−6.09	2.91	PD	12.92	5.56
					WH11	65.51	9.04	−4.99	0.54	A	7.99	4.45
	qKL9-3	umc1519-umc1231	65.7–68.1	6.36	ES11	67.11	9.05	−6.59	−0.71	A	12.62	8.03
	qKL9-4	umc1494-umc2346	70.1–85.6	10.61	WH11	74.81	9.05	−4.51	−0.68	A	6.44	3.78
KW	*qKW1-1*	umc2225-bnlg1007	58.5–63.6	12.55	ES11	62.01	1.02	−5.73	2.21	PD	16.06	9.62
	*qKW1-2**	bnlg1007-bnlg439	63.62–77.88	16.67	ES12	67.61	1.02	−3.92	−0.24	A	9.55	7.93
					HG11	69.51	1.03	−4.66	1.61	PD	15.63	9.00
					ES11	69.51	1.03	−5.72	1.66	PD	17.75	11.31
					HG12	69.51	1.03	−5.76	1.06	A	20.51	12.38
					WH11	72.51	1.03	−4.45	2.35	PD	14.17	6.86
	qKW2-1	umc2245-umc1227	19.6–23.6	1.66	WH11	21.71	2.01	4.02	−1.87	PD	10.76	5.51
	*qKW2-2**	bnlg1831-bnlg1138	79.7–82.3	20.13	ES11	81.01	2.05	4.17	0.43	A	8.88	11.38
					ES12	81.02	2.05	4.55	−1.03	PD	12.83	10.60
	qKW2-3	umc2023-umc1890	83.8–91	22.18	ES12	84.91	2.07	4.25	−0.49	A	11.86	10.66
					ES11	86.91	2.07	4.44	0.67	A	10.78	12.07
	qKW2-4	umc1946-umc2625	93.9–97	9.37	HG11	94.81	2.07	2.58	0.34	A	5.15	5.65
					ES12	95.81	2.07	2.49	1.13	PD	4.36	6.58
					ES11	96.81	2.07	3.13	1.65	PD	5.72	9.6
	*qKW2-5**	bnlg1662-bnlg1316	102.7–111	6.51	HG11	107.71	2.08	3.04	0.49	A	7.38	7.77
	qKW2-6	bnlg1316-umc1464	112–115.3	1.46	HG12	112.02	2.08	2.83	0.76	PD	5.58	6.97
	qKW2-7	umc1526-umc1230	117.1–130.4	15.20	HG12	122.31	2.08	3.34	0.76	PD	7.77	7.84
					HG11	123.31	2.08	3.4	−0.49	A	9.07	6.85
	qKW3	umc1320-umc1273	87–102.15	0.23	HG12	88.91	3.08	2.13	2.03	D	2.63	3.5
					HG11	98.21	3.08	1.56	1.89	OD	1.7	3.1
	qKW4-1	umc1667-umc2041	81.1–88.2	6.15	HG11	84.31	4.08	−3.69	2.48	PD	9.37	5.12
					ES12	87.51	4.08	−3.92	0.41	A	9.36	7.45
					WH11	87.51	4.08	−3.26	0.18	A	7.1	5.02
	*qKW4-2**	umc1051-bnlg292b	91.4–99.8	22.39	HG11	93.41	4.08	−3.43	1.42	PD	8.04	4.52

Table 4 continued

Trait	QTL[a]	Marker interval	Range (cM)	Size (Mb)[b]	Env.[c]	Peak position (cM)	Bin[d]	*A*[e]	*D*[f]	Gene action[g]	PVE[h] (%)	LOD
					ES11	96.41	4.08	−4.73	0.12	A	11.85	9.91
					WH11	95.39	4.08	−3.28	0.17	A	7.41	5
	*qKW5**	umc2294-umc2161	62–68.2	47.10	ES11	67.01	5.03	2.6	0.05	A	3.91	3.62
	*qKW6**	phi070-umc2165	106.68–115.94	2.18	ES12	110.81	6.07	2.18	0.29	A	3.19	2.92
	*qKW9-1**	umc2084-bnlg1583	10.9–20.2	5.08	HG12	17.91	9.01	−2.15	−1.48	PD	2.74	3.71
	qKW9-2	umc2346-umc1714	94.3–114.1		HG11	108.01	9.07	−3.23	0.68	PD	7.26	4.14
KT	*qKT1-1**	umc1568-umc1403	64.8–70.8	17.20	ES11	67.61	1.02	−3.15	0.29	A	12	9.59
					ES12	67.61	1.02	−3.07	−0.78	PD	10.79	14.2
	*qKT1-2**	umc1403-umc2171	76.1–79	18.53	ES11	77.91	1.03	−4.47	0.94	PD	11	5.95
					ES12	77.91	1.03	−2.61	−0.37	A	6.02	6.72
	*qKT1-3**	umc1144-bnlg1811	85.6–89.6	6.48	WH11	85.81	1.04	−3.13	−0.68	PD	5.47	4.48
					ES11	85.81	1.04	−2.68	0.45	A	5.64	3.65
					ES12	87.81	1.04	−1.68	−0.58	PD	3.09	4.53
					HG11	88.81	1.04	−4.13	0.14	A	14.93	10.01
	*qKT1-4**	bnlg2086-umc1323	94.8–98.7	86.83	ES11	95.71	1.04	−2.27	0.17	A	5.07	3.73
					HG12	96.01	1.05	−3.5	−0.2	A	11.36	7.81
					ES12	96.01	1.05	−2.18	−0.39	A	5.85	6.45
					WH11	97.01	1.05	−4.09	−1	PD	8.81	6.44
					HG11	98.01	1.05	−4.7	−0.4	A	17.98	13.87
	qKT1-5	umc2234-umc1335	101.9–109	10.00	ES12	103.21	1.06	−1.6	−0.7	PD	3.23	5.23
	qKT2	mmc0111-umc1422	42.2–61.3	5.57	ES12	49.41	2.02	2.07	−1.26	PD	6.66	5.03
	*qKT4-1**	umc1791-umc2027	65–66.7	58.22	HG12	66.51	4.06	−1.71	−1.98	D	2.83	5.94
	qKT4-2	umc1899-umc2135	87.3–97.8	17.59	HG11	87.01	4.08	−2.15	−0.85	PD	3.42	4.9
					ES11	90.21	4.08	−1.58	−0.66	PD	2.93	4.36
					ES12	92.31	4.08	−1.32	−0.3	PD	2.61	3.92
	qKT5-1	umc1496-umc1761	32.3–56.4	12.58	WH11	42.91	5.01	1.98	−6.14	OD	2.14	4
	qKT5-2	umc1761-umc2294	55.2–65.7	19.56	HG11	62.21	5.03	−1.49	−1.26	D	1.87	3.58
	*qKT5-3**	umc1784-umc1747	66.9–71.4	83.29	WH11	68.21	5.03	7.42	−3.24	PD	13.68	4.05
	qKT8-1	umc1075-umc1974	19–44.2	9.99	ES12	37.41	8.02	1.8	−0.42	PD	5.46	5.47
	*qKT8-2**	umc2052-umc1638	111–120.1	3.18	HG11	119.11	8.08	−1.69	−1.11	PD	2.47	4.86
	qKT9-1	umc1893-umc2370	52.3–59.3	11.46	HG12	54.11	9.03	2.61	−0.03	A	6.12	3.94
					ES12	54.11	9.03	2.45	−1.11	PD	9.68	6.78
					ES11	56.11	9.03	2.45	−0.21	A	7.87	6.2
	*qKT9-2**	bnlg1159-umc1494	61.4–69.4	25.13	ES12	61.71	9.04	2.03	−0.88	PD	6.82	5.09
					ES11	67.41	9.05	2.55	−0.2	A	8.26	6.38
	qKT10-1	phi059-umc1863	30.1–34.8	6.96	ES11	30.71	10.02	1.02	1.02	D	1.42	4.47

Table 4 continued

Trait	QTL[a]	Marker interval	Range (cM)	Size (Mb)[b]	Env.[c]	Peak position (cM)	Bin[d]	*A*[e]	*D*[f]	Gene action[g]	PVE[h] (%)	LOD
					ES12	30.71	10.02	0.7	1.29	OD	0.84	5.6
	*qKT10-2**	umc1785-bnlg1655	38–43	60.69	ES12	40.31	10.03	0.82	0.78	D	0.98	3.68
					HG12	41.71	10.03	2.28	0.67	PD	4.22	5.09
					HG11	42.71	10.03	1.26	0.85	PD	1.12	3.2
	qKT10-3	umc1911-umc1930	47–52.6	12.47	HG12	47.31	10.04	2.25	1.04	PD	4	5.38
HKW	*qHKW1-1*	bnlg1014-umc1222	34.4–45.2	4.50	HG12	39.31	1.01	−0.93	0.28	PD	7.33	4.11
	qHKW1-2	umc1727-umc2224	41.33–52.6	3.61	ES12	48.21	1.01	−1.45	0.09	A	5.82	3.43
					HG12	49.21	1.01	−0.87	0.21	PD	5.73	3.1
	qHKW1-3	umc2224-umc1568	56.4–58.5	3.64	ES12	58.31	1.02	−1.82	0.4	PD	7.41	3.41
	*qHKW1-4**	bnlg1007-bnlg439	63.5–77.88	16.67	ES11	68.51	1.03	−1.79	0.55	PD	12.23	6.82
					WH11	73.51	1.03	−0.97	0.66	PD	10.03	4.69
	*qHKW1-5**	umc1144-bnlg2086	81.2–90.7	19.37	HG12	85.81	1.04	−1.16	0.69	PD	12.02	5.62
					HG11	88.81	1.04	−1.13	0.15	A	12.2	6.68
	qHKW1-6	umc1323-umc2234	98.9–101.5	22.03	HG11	100.51	1.06	−1.24	0.11	A	12.8	7.52
	qHKW1-7	umc2151-umc1356	105.9–109	13.91	HG11	107.91	1.06	−1.08	0.36	PD	10.21	5.2
	qHKW2-1	umc1165-umc1265	19.5–25.1	1.37	HG11	21.81	2.02	0.51	0.2	PD	2.09	2.63
					ES11	21.81	2.02	0.67	0.67	D	1.64	4.19
	qHKW2-2	bnlg1831-bnlg1138	80.3–83.3	20.13	WH11	81.01	2.05	0.79	−0.12	A	6.43	5.13
	*qHKW2-3**	umc2023-umc1890	86.3–95.7	22.18	ES11	90.91	2.07	1.31	0.91	PD	6.72	10.57
					ES12	90.91	2.07	1.42	1.2	D	6.83	8.98
	qHKW2-4	umc1049-bnlg1316	97.5–112.2	8.02	ES12	100.11	2.08	1.15	0.66	PD	4.84	6.98
					HG11	107.71	2.08	0.4	0.7	OD	1.6	5.64
	qHKW2-5	umc1526-umc1230	115.3–123.5	15.20	HG11	120.31	2.08	0.4	0.47	D	1.55	3.2
	*qHKW4**	umc1847-umc1667	78.7–87.03	7.48	ES11	83.11	4.07	−1	0.09	A	3.84	2.97
	*qHKW5**	umc2060-umc1784	63.5–69.4	47.59	ES11	66.51	5.03	0.78	0.54	PD	2.43	3.88
	*qHKW7**	bnlg2259-umc2197	93.3–117.3	7.40	ES12	102.31	7.05	0.04	1.45	OD	0.46	3.68

[a] The names followed by * were QTL simultaneously detected by joint QTL analysis among all environments

[b] Physical size of marker interval

[c] Environment. WH11, HG11 and ES11 represent Wuhan, Huanggang and Enshi in 2011, respectively; HG12 and ES12 represent Huanggang and Enshi in 2012, respectively

[d] The specific genetic region included the peak position of QTL in differential environments. Bins in maize are designated with the chromosome number followed by a two-digit decimal (e.g., 1.00, 1.01, 1.02, etc.) (http://www.maizegdb.org/cgi-bin/bin_viewer.cgi)

[e] The additive effect of the QTL, with negative effect was contributed by V671 and positive effect was contributed by Mc; the unit of KL, KW and KT was millimeter (mm) and HKW was measured in gram (g)

[f] The dominant effect of the QTL

[g] A, D, PD, and OD represent additive, dominance, partial dominance, over-dominance effect, respectively, based on Stuber et al. (1987)

[h] The percentage of phenotypic variation explained by corresponding QTL

QTL together in single environment ranged from 16.46 to 40.45 %. Two of the six QTLs identified across two environments, *qHKW1-4* and *qHKW1-5*, had major effect on HKW with phenotypic variation explained >10 %. All QTL for HKW co-located with QTL for kernel-size traits except for *qHKW7*, indicating the close genetic correlation between kernel size and kernel weight which may result from pleiotropy or 'multifactorial linkage'. Ten QTLs for HKW were characterized by A or PD effects, whereas the rest five QTLs with minor phenotypic variation explained showed dominance effects (D or OD).

QEIs

There were 28 putative QTLs associated with 4 kernel-related traits that were detected by joint analysis with MCIM method; of these, 23 QTLs exhibited significant coincidence with the results of single-environment mapping (Table 4 and Supplementary Table S2). For KL, KW, KT and HKW, all significant QTL identified through MCIM method could explain 4.32, 31.12, 31 and 36.61 % of phenotype variance, respectively. Most of these QTLs were detected with additive main effect, while six of them were involved in significant QTL × environment interaction (QEI) (Table 5) through joint analysis. Of which, two QTLs for KT have significant dominant × environment interactions ($P < 0.05$), while one of them, *qKT1-3* has additive × environment interaction ($P < 0.05$) simultaneously. This suggests that QTL for KT may be modified by environmental conditions. Other four QTLs were detected just with significant additive × environment interactions ($P < 0.05$). The additive × environment interactions for the target traits were responsible for 0.22–1.75 % of the heritability, while dominant × environment interactions contributed less, with 0.18–0.43 %. In addition, the three environment-specific QTLs with QEI, *qKW5*, *qKT5-3* and *qHKW5* were identified in overlapped genomic regions on chromosome 5, indicating that the interactions of this region with environments might involve in the influence on kernel development.

Epistatic interaction

A total of five significant epistatic interaction loci ($P < 0.05$) for KW and KT were identified with additive-by-additive interaction, additive-by-dominance interaction, dominance-by-additive interaction or dominance-by-dominance interaction effects (Table 6). Four epistatic interactions occurred within the genetic regions of significant QTL, while the other one was distinct between significant QTL and a non-significant locus on chromosome 8. Although none of these epistatic interactions were consistent in different environments, four referred loci, umc1403–bnlg439, phi448880–umc1714, bnlg1662–umc2005 and umc2135–bnlg292b, were repeatedly detected as involved in two epistatic interactions, even for different traits like umc1403–bnlg439 which was responsible for two major QTL, *qKW1-2* and *qKT1-1*, in different interaction pairs—indicating that epistasis at this genomic region may participate in regulating maize kernel size. The phenotypic variation of target traits explained by epistatic interactions was less than main effects of relevant QTL, indicating that main effect of QTL may play an essential role in determining maize kernel size and weight.

Clusters with co-located QTL for kernel-related traits

In the overview of the identified QTL in this study, 8 QTL clusters comprising 34 QTLs were scattered on chromosomes 1, 2, 4, 5 and 9 (Tables 4, 7; Fig. 2). Half of the clusters with co-located QTL for three traits spread on chromosomes 1, 2, 4 and 5; while the other four clusters on chromosomes 1, 2 and 9 encompassed QTL for two kernel-related traits. Among the aforementioned clusters, QTLs for HKW were always detected together with QTL for kernel size apart from QTL cluster VIII where only

Table 5 QTL × environment interactions influencing kernel-related traits in different environments

Trait	QTL	AE2[a]	AE3[a]	AE4[a]	AE5[a]	DE1[b]	H^2(ae)[c]	H^2(de)[d]
KL	*qKL2-1*	−2.46**					0.0035	
KW	*qKW5*		1.27*				0.0069	
KT	*qKT1-3*			0.96*		−1.46*	0.0022	0.0018
KT	*qKT5-3*					−2.14***		0.0043
HKW	*qHKW2-3*		0.80***	−0.82***	0.79***		0.0175	
HKW	*qHKW5*		0.60**	−0.62**	0.55**		0.0128	

[a] AE is the additive by designated environment interaction effect

[b] DE is the dominance by designated environment interaction effect

[c] H^2(ae) is heritability of the additive by designated environment interaction effect

[d] H^2(de) is heritability of the dominance by designated environment interaction effect

Table 6 Digenetic epistatic QTL for maize kernel-related traits identified in $F_{2:3}$ families under different environments

Env	Trait	QTL_i	interval_i	QTL_j	interval_j	AA[a]	AD[a]	DA[a]	DD[a]	H^2(aa)[b]	H^2(ad)[b]	H^2(da)[b]	H^2(dd)[b]
HG11	KW	*qKW1-2*	umc1403-bnlg439	*qKW9-2*	phi448880-umc1714		−2.97*	3.38*	−4.71*		0.0106	0.0071	0.0106
	KW	*qKW2-5*	bnlg1662-umc2005	*qKW9-2*	phi448880-umc1714			3.48**	−4.45*			0.001	0.0096
ES11	KW	*qKW2-5*	bnlg1662-umc2005	*qKW4-2*	umc2135-bnlg292b		−2.90*		−4.30*		0.0034		0.0087
	KW	*qKW4-2*	umc2135-bnlg292b	-/chr.8	umc1069-umc1638			3.65**				0.0002	
	KT	*qKT1-1*	umc1403-bnlg439	*qKT1-3*	umc2171-umc1144	17.04***	−19.98***	17.63***	−17.96***	0.0007	0.0013	0.005	0.0154

-/chr.8, a non-significant quantitative loci on chromosome 8, but involved in epistatic interaction

*, ** and *** indicate significant level at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively

[a] AA, AD, DA and DD are the additive-by-additive, additive-by-dominant, dominant-by-additive and dominant-by-dominant epistatic interaction effects, respectively

[b] H^2(aa), H^2(ad), H^2(da) and H^2(dd) are the heritability of the additive-by-additive, additive-by-dominant, dominant-by-additive and dominant-by-dominant epistatic interaction effects, respectively

some QTLs for KL and KT were co-located. The opposite parents conferring positive alleles of co-located QTL for KL and KT may account for the no significance for HKW in QTL cluster VIII and the negative correlation between them, while the positive alleles of co-located QTL in other seven QTL clusters were consistently contributed by the same parent. The series of QTL clusters in the present study indicated that the underlying genetic correlation and pleiotropic effect may influence maize kernel-related traits. Furthermore, multiple kernel-related traits are likely to be simultaneously improved.

Discussion

Complex genetic basis of QTL detected for kernel-related traits

In this study, there was a wide variation of kernel-related traits in the $F_{2:3}$ families derived from two parents with contrasting kernel-related traits: Mc (small kernel) and V671 (large kernel). The close correlation among kernel-related traits and the relatively high broad-sense heritability indicated stable phenotypic and genetic association between kernel size and kernel weight. In term of QTL mapping results, the disparate numbers of significant QTL per trait which ranged from 6 for KL to 18 for KT as well as an asymmetric and clustered distribution among genomic regions revealed the complex nature of the kernel-related traits. Besides, the phenotypic variation explained by stable QTL also differed in magnitude among different environments, as a result of interaction with environments (Xu 2010). At the same time, some environment-specific QTL for target trait also accounted for relatively large proportions of phenotypic variation. This complex phenomenon may be due to context-dependent effects and regulation of minor polygenes (Mackay et al. 2009). According to Malosetti et al. (2008) and Messmer et al. (2009), the positions and stability of QTL as well as the direction and magnitude of genetic effects on target traits were not exactly predicted by QTL analysis in single environment. However, QTL that has obvious genetic effect could be preferentially identified in varying environments. In this study, twelve significant QTLs contributed over 10 % of phenotypic variation and were mapped in multiple environments; ten of them were found by both single-environment QTL analysis and joint analysis. For example, two robust QTLs with best repeatability, *qKW1-2* and *qKT1-4*, were detected in all five environments. Moreover, more than half of all detected loci were environment-specific QTL, indicating that a complex genetic constitution with major and minor effects controlled maize kernel-related traits. The complex genetic basis of kernel-size traits and kernel weight was

Table 7 Characterization of QTL clusters for four maize kernel-related traits identified in $F_{2:3}$ families across multiple environments

QTL cluster no.	Chr.	Trait included	Marker interval	Physical position (bp)	Bin	Positive alleles[a]	Rang of explained phenotypic variation (%)
I	1	KW + KT + HKW	bnlg1007-bnlg439	26648076–43864136	1.02–1.03	V671 + V671 + V671	6.02–20.51
II	1	KT + HKW	umc1144-umc1335	64259586–196926685	1.04–1.06	V671 + V671	3.09–17.98
III	2	KW + HKW	umc1165-bnlg1227	3945514–4688370	2.01–2.02	Mc + Mc	1.64–10.76
IV	2	KL + KW + HKW	umc1884-umc1890	107384428–190855347	2.05–2.07	Mc + Mc + Mc	2.12–11.86
V	2	KW + HKW	umc2005-umc1464	206227032–213768587	2.08	Mc + Mc	1.54–9.07
VI	4	KW + KT + HKW	umc1194-umc2135	177331242–201133111	4.07–4.08	V671 + V671 + V671	2.36–11.86
VII	5	KW + KT + HKW	umc2294-umc2161	33097126–74217977	5.03	Mc + Mc + Mc	2.4–13.68
VIII	9	KL + KT	umc1634-umc1519	23407901–123304894	9.03–9.04	V671 + Mc	1.26–12.62

Chr. represents chromosome

[a] V671: parent with large kernel; Mc: parent with small kernel; according to the additive effect of QTL in the same cluster, each parent responsible for increasing phenotype was listed as the order of 'trait included'

also reflected on the gene action of QTL: A, PD, D and OD were all shown. Most QTL (42/55) expressed A or PD effect across different environments, especially the major QTL. These results demonstrated that additive effects and partial dominance effects may play important roles in controlling the development of maize kernel size and weight. The large-kernel parent V671 contributed to increasing effects for 27 QTL (49.09 %) including 10 of the 12 significant major QTL mentioned above, meaning that V671 is a good donor for improving maize kernel-related traits. Whereas, the small-kernel parent Mc contributed to the other 28 QTLs (50.91 %), embracing the last two major QTL and other QTL which were found in several environments explained >5 % of phenotypic variation. Obviously, the QTL alleles from parent with low values on favored traits also played an important role in increasing phenotypic value. Therefore, alteration in direction of parental increasing alleles would be a critical component of QTL assessment and valuable in illuminating the genetic basis of kernel yield.

QTL clusters associated with multiple kernel-related traits

One of the central concepts in genetical genomics is the existence of QTL clusters, in which widespread downstream changes in expression of genes result from a distant single polymorphism that located in the same genomic regions (Schadt et al. 2003). Associative traits are prone to share regions with significant QTL (Austin and Lee 1996, 1998; Li et al. 2007). Domestication has increased the size of maize kernels compared to its progenitor teosinte. In rice, QTL of domestication-related traits tends to form clusters that coincide with the regions harboring favorable genes (Cai and Morishima 2002). In an overview of QTL distribution in the present study 34 QTLs were clustered in 8 genetic regions on chromosomes 1, 2, 4, 5 and 9 (Tables 4 and 5) including all 12 robust major QTLs.

As demonstrated through multi-environmental trials in previous studies (Li et al. 2013; Peng et al. 2011; Veldboom and Lee 1996), we also found that KL was controlled by several genetic loci on chromosome 9, which have also been reported to significantly influence kernel yield (Li et al. 2009, 2013). KL in our study was primarily regulated by the major QTL *qKL9-1* (bin 9.02–9.03) and *qKL9-2* (bin 9.04) in cluster VIII, and co-localized with QTL *qKT9-1* (bin 9.03) and *qKT9-2* (bin 9.03) for KT, respectively. QTLs in this cluster were only for KL and KT with no significant effect on HKW, suggesting a tension or trade-off between the two kernel developmental dimensions that would both benefit from assimilates. The genomic region of QTL cluster VIII also harbored co-localized QTL for yield and its component traits in other studies.Austin and

Lee (1998) detected a QTL at bin 9.03, stably influencing 300-kernel weight in stress and non-stress environments. In three different recombinant inbred lines (RILs) among six environments Li et al. (2013) identified co-located QTL for kernel length, width and yield on bin 9.03. In the genetic region of bin 9.04, Peng et al. (2011) detected a stable major QTL for grain yield at bin 9.04 in an $F_{2:3}$ population across multi-environments. This region was also frequently reported to be involved in the QTL for kernel weight in other studies (Austin and Lee 1998; Lu et al. 2006). Therefore, bins 9.03 and 9.04 are noteworthy for genetic improvement of maize kernel size and yield.

Another QTL for KL, *qKL2-2* clustered with *qHKW2-2*, *qHKW2-3*, *qKW2-2*, *qKW2-3* and *qKW2-4* in bin 2.05–2.07 on chromosome 2 (cluster IV), which simultaneously influenced HKW, KW and KL. The small-kernel parent Mc contributed increasing alleles for all these significant loci. Around this genetic region, Li et al. (2013) found a QTL for KW on bin 2.07 clustered with three QTLs each for KL, KT and grain yield, identified by several RILs among multi-environments. QTL for kernel weight under water-stressed conditions (Lu et al. 2006) and kernel volume (Peng et al. 2011) were also identified on bin 2.07. These QTLs in bin 2.05–2.06 seem to indicate a novel genomic region for kernel-related traits.

QTLs for KW and HKW were co-located in another two clusters (III and V) on chromosome 2 (bins 2.01–2.02 and 2.08). Both single-environment and joint QTL analyses identified *qKW2-2* and *qHKW2-3* (bin 2.08) with moderate phenotypic contributions. A QTL for kernel width on bin 2.08 was also revealed with moderate contribution (<10 %) in specific environments (Peng et al. 2011), as were QTL in cluster III. No QTL for KW on bin 2.01–2.02 has been detected with any confidence in the past, but several yield components, like cob diameter, restricting kernel development, were reported previously (Austin and Lee 1996), ear length (Lu et al. 2011), semi-diameter for cob and ear (Li et al. 2009), seemed to be controlled by efficient loci around this genomic region.

Clusters VI and VII, both with multiple QTL, simultaneously facilitated KT, KW and HKW—and were mapped on chromosomes 4 (bin 4.07–4.08) and 5 (bin 5.03), respectively. Major QTLs *qKW4-1*, *qKT4-2* and *qKW4-2* co-located on bin 4.08 were stably detected, where Li et al. (2013) also found clustered QTL for KW and KT in several RIL populations. A series of previous studies reported numerous QTL on bin 4.08 for kernel weight (Lu et al. 2006; Veldboom and Lee 1996) and yield components influencing kernel shape: such as kernel row number (Austin and Lee 1996; Lu et al. 2011), semi-diameter of ear (Li et al. 2009) and ear length (Lu et al. 2006). *ZmGW2-CHR4* and *ZmGW2-CHR5* are two maize homologs of *GW2*, which controls grain width and weight in rice (Li et al. 2010a). *ZmGW2-CHR4* was demonstrated significantly influencing kernel weight and located just in the genetic region of *qKW4-2*. A QTL focus on grain yield was identified on bin 5.03–5.04 with 256 $F_{2:3}$ families in five environments (Lima et al. 2006). In the present study, all the three QTLs in cluster VII (bin 5.03) were environment-specific with relative less stable influence on kernel size and weight.

Two more notable QTL clusters, I (bin 1.02–1.03) and II (bin 1.04–1.06) on chromosome 1, possessed several significant QTL for kernel-related traits. QTL cluster I consisted of a range of QTL affecting KW, KT and HKW, while QTL in cluster II regulated KT and HKW. Two major QTLs, *qKW1-2* (bin 1.03) and *qKT1-4* (bin 1.05), were solidly identified in all environments with the corresponding highest effects of up to 20.51 and 17.98 %, respectively, strongly implying their determining effect on phenotype and the presence of kernel trait-related genes. The majority of other QTL in these two clusters, including major QTL *qKT1-4* (bin 1.04), was also detected as stably influencing maize kernel development across different agro-ecological environments. Veldboom and Lee (1996) published a suite of QTL for grain yield, including such yield components as kernel length, around the genetic regions of cluster I in an F_3 population. The importance of bin 1.04 on maize kernel weight and other yield components was verified by Austin and Lee (1996, 1998) in a RIL population derived from Mo17 and H99. In recent years, QTLs on bins 1.03 and 1.04–1.06 with obvious contributions to grain yield and kernel size and weight were discovered in $F_{2:3}$ (Li et al. 2007; Peng et al. 2011; Ribaut et al. 1997), BC_2F_2 (Li et al. 2007) and RIL populations (Li et al. 2012; Messmer et al. 2009) under different experimental environments. Within the abundant candidate genes, *ZmGS3* as a putative *GS3* ortholog located between *qKT1-3* and *qKT1-4* and involved in maize kernel development was successfully cloned and characterized by Li et al. (2010b). *ZmGS3* with influence on KW and HKW but not KT and with different functional polymorphisms from rice *GS3* imply that other relevant genes regulating maize kernel size with distinctive mechanisms must remain concealed. Collectively, the adjacent genetic regions of bins 1.03 and 1.04–1.06 should have great potential for improving maize kernel-related traits and kernel yield.

The genetic regions aforementioned with clustered/co-located robust QTL are worth of further investigation due to the importance of genetic control of kernel development as well as grain yield. Peng et al. (2011) reported seven major QTLs on chromosomal regions responsible for maize kernel-related traits and yield components in two $F_{2:3}$ populations. Although studies were conducted in the same generation, none of the major QTL was coincident with the results of the present study, possibly due to the

different genetic background. According to Li et al. (2013), one of the seven important QTL clusters located on bin 4.08, for kernel-related traits and yield components in RIL populations was in agreement with the result of this study. Through QTL detection and meta-analysis in three populations ($F_{2:3}$, BC_2F_2 and RIL), three main genetic regions of bins 7.02–7.03, 1.03–1.04 and 10.05–10.06 were identified for maize kernel development (Li et al. 2012). Most of the QTL clusters identified in the present study would be novel loci regulating kernel-related traits in maize, especially the five optimal QTL clusters in the genetic regions of bins 1.02–1.03, 1.04–1.06, 2.05–2.07, 4.08 and 9.03–9.04. Remarkably, the physical distance of the most QTL clusters were too large to isolate candidate genes, and it was more expansive of the QTL clusters near or across chromosomal centromere, which makes it necessary to conduct fine-mapping of the major QTL in cluster regions with enriched markers. However, these crucial clusters provide more opportunities to identify important agriculturally beneficial genes underlying these genetic regions. The linked or co-located QTLs are inferred to benefit from the association of adaptive phenotypes during domestication and will lead to a cumulative increase in kernel yield due to the integrative positive effect (Marathi et al. 2012). Pleiotropic regulator(s) or 'multifactorial linkage' of multiple traits offers selective advantages and provides a rational explanation for the numerous clustered QTL across the genome.

Stability of QTL for kernel-related traits among environmental trials

The stability of significant genetic regions influencing kernel-related traits was displayed well by comparing the joint QTL mapping with single-environment QTL analysis, as recommended by Messmer et al. (2009) and Malosetti et al. (2008). Of the 23 consistent QTLs, twelve were stably identified with the same direction of increasing alleles among multiple environments: one for KL (*qKL9-2*), three for KW (*qKW1-2*, *qKW2-2* and *qKW4-2*), five for KT (*qKT1-1*, *qKT1-3*, *qKT1-4*, *qKT9-2* and *qKT9-2*) and three for HKW (*qHKW1-4*, *qHKW1-5* and *qHKW2-3*). However, the other consistent QTLs were basically significant loci with relatively lower phenotypic contributions, suggesting that kernel-related traits in maize appeared to be controlled by some major QTL and a large number of minor-effect QTL identified in specific environments or locations (Li et al. 2013). QEI may epitomize the existence of environment- or location-specific QTL which lacked stability or main effects among varied environments/locations (Cho et al. 2007; Hittalmani et al. 2003; Messmer et al. 2009). Meanwhile, QTL with major effect does not mean lack of QEI. This is supported by the fact that QEI was also detected for some repeatedly identified QTL (Hosseini et al. 2012), resembling *qKT1-3*, a major KT QTL, as well as one of the nine location-specific QTL, *qHKW2-3*; and by the fact that QTL effects estimated in multiple environments could be very different. For target traits, those QEIs were detected accounting for much less phenotypic variation than that explained by QTL through joint analysis, as a consequence, they did not appreciably alter the main effects of QTL either by magnitude or direction. Peng et al. (2011) reported that QTLs for kernel-related traits were more consistent across environments and genetic backcrosses than QTL of grain yield influenced by QEI. Similar results were found in eleven RILs derived from one common parent by Li et al. (2013). Grain yield in maize might be regulated by a large number of minor-effect QTLs that are sensitive to environment (Beavis et al. 1994). In addition, the majority of QTL for grain yield presented instability and less co-localization across diverse water regimes, locations, years or cropping seasons in several studies (Austin and Lee 1998; Li et al. 2013; Lima et al. 2006; Lu et al. 2006; Messmer et al. 2009; Ribaut et al. 1997). Yield component traits including kernel size and weight displayed more advantages for genetic improvement. Improving kernel size and weight is such an efficient strategy for increasing grain yield, which has been demonstrated by related research in rice.

As members of the grass family (*Poaceae*), maize and rice share good synteny of genomes and most gene families are the same (Schnable et al. 2009), and important agronomic and domestication-related QTLs were revealed in orthologous regions (Yan et al. 2004). Do the maize orthologs of isolated rice grain genes coincide with the stable major QTL in our study that also have an important influence on maize kernel size and yield? There are limited results available on this question. One of the two orthologs of the *SPL14* (Miura et al. 2010), which controls panicle branching and grain production of rice, lays in the prominent genetic region on chromosome 4 where *qKW4-2* and *qKT4-2* were co-located, and both were stably expressed among multi-environments. Two co-located QTLs for KL, *qKL9-2* and *qKT9-2*, on chromosome 9 contain one of the two orthologs of another rice yield-related gene, *APO1* (Terao et al. 2010). However, the influence on maize kernel size and yield of the above two candidate orthologs requires further investigation. Although the cloned homologs *ZmGS3* and *ZmGS2* were the only two maize orthologs confirmed as involved in maize kernel development, they were marginally associated with maize kernel size and just adjacent to our major genetic regions. Orthologs of cloned rice yield-related genes seem to change or reduce their function in maize, indicating the underlying of the major QTL in the present study, maize kernel size and weight were controlled by distinct genetic mechanisms or other members of the same gene families found in rice grain yield and grain development.

These stably identified QTL could contribute to effective selection of genotypes with broad adaptation across diversity agro-ecological conditions. Meanwhile, the abundant environment-specific QTL, particularly the consistent ones among two mapping strategies, would allow more opportunities to understand the importance and genetic basis of QEI. As interaction with environment is the natural of creatures, QEI significantly associating with plant performance cannot be ignored during breeding for stability and adaption, especially for resource-limited environments. To increase crop productivity, there were two strategies dealing with QEI in the breeding program. First, identification of some stable robust QTL or QTL with minor QEI should be highly desirable. Second, development of widely adapted cultivars by pyramiding some stable QTL and/or cultivars with specific adaption by pyramiding stable major QTL and reliable environment-/location-specific QTL together might be very useful for optimizing MAS of kernel yield.

Epistatic interactions between QTL

For complex quantitative traits, the interaction effects between loci/genes, or epistasis have been considered as essential to understanding genetic regulation (Carlborg and Haley 2004; Phillips 2008). The reduced genetic heterogeneity after crosses and the missing proportion of phenotypic variation explained by the identified QTL, compared to the heritability of a certain trait, could be partly due to epistasis (Doebley et al. 2006; Mackay et al. 2009; Miedaner et al. 2011; Phillips 2008; Reif et al. 2011; Xu and Jia 2007). Although the effect of epistasis may not be significant in different crops and traits, these interactions could be selected and retained to impact on the phenotype of target traits (Würschum 2012), and will obviously influence the efficiency and accuracy of marker-assisted breeding (Carlborg and Haley 2004; Steinhoff et al. 2012). Particularly, epistatic interaction as an important regulator for maize grain yield and its components has been often detected between non-significant major loci (Ma et al. 2007; Peng et al. 2011). In the present study, five pairs of epistatic interactions regulating KW and KT were detected, mostly among significant QTL, indicating that these significant loci could influence each other's genetic background while controlling kernel size. Properly pyramiding this epistasis QTL may improve kernel yield (Wang et al. 2012a). Recently, Wang et al. (2012b) showed that the genetic interaction between two rice seed-size genes, *OsSPL16* and *GS3*, led to improved grain yield and quality through simultaneously targeting *GS3* and *OsSPL16* within a marker-assisted strategy. Zhao et al. (2011) found that the pyramiding effect could be obvious among QTL for the rice grain length, with epistasis occurring only when the direction of epistatic effects was the same as the additive effect of target QTL/genes. In addition, there was an epistatic effect even between closely linked QTL (Kroymann and Mitchell-Olds 2005; Mackay et al. 2009). Among the five pairs of epistatic interactions observed in the present study, four significant QTLs, *qKW9-2*, *qKW2-5*, *qKW4-2* and *qKT1-1*/*qKW1-2* were involved in two pairs of interactions, that is, they co-operated with two different loci either for different traits or in different environments, suggesting that these loci may be regarded as epistatic regulators prone to taking part in multiple binary interactions—similar results were previously observed (Reif et al. 2011; Würschum 2012).

As a focal point for the unification of many traditionally research areas that used to be disparate, epistasis of disparate loci is important for elucidating the functional nature of complex genetic system and the long-term change of biological evolution (Phillips 2008). To explore and clarify the potential epistatic network and the genetic regulation of complex quantitative traits, large population sizes, high-throughput screens from DNA level to phenotype and rigorous analysis of gene interaction with good bioinformatic tools will be required in the future (Carlborg and Haley 2004; Phillips 2008). These tools will enable the pursuit of epistatic QTL and confirmation of the epistatic effect on maize kernel size and yield in efficient pyramiding through MAS.

Conclusion

In the present study, the estimates of phenotype and genotype of $F_{2:3}$ families in multiple agro-ecological circumstances reveal the significant association between kernel size of KL, KW and KT and kernel weight. Numbers of QTL dramatically influencing kernel size and weight with additive and partial dominance effects were co-localized in eight genomic regions (bins 1.03, 1.04–1.06, 2.01–2.02, 2.05–2.07, 2.08, 4.08, 5.03 and 9.03–9.04). The integrative positive effects of the clustered QTL will lead to a cumulative increasing kernel yield due to the improved kernel size and kernel weight. Meanwhile, the pleiotropic effect of enriched QTL and genetic epistasis between two genomic regions may play an important role in mutual interactions of these kernel-related traits. The information generated in this study could well aid in understanding the genetic basis of maize kernel-related traits and fine-mapping genes underlying the robust major QTL in the optimal clusters (bins 1.03, 1.04–1.06, 2.05–2.07, 4.08 and 9.03–9.04). However, challenging research questions remains such as how to accurately estimate the 'real' breeding value of a significant QTL for kernel-related traits without genetic background interference and how to best clone and transfer

the robust QTL to other populations. And due to the limited resolution in the present study, there could be hundreds or thousands of genes underlying the major QTL. Therefore, further QTL validation will be proceeding with advanced backcross population (QTL-NIL or chromosomal segment substitution lines). Besides, support from other omics researches, like transcriptomics, should also be considered to promote genetic analysis of kernel development and the isolation of favorable alleles for molecular breeding of high-yield maize based on this study.

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Conflict of interest The authors declare that they have no conflict of interest.

Ethical standards The experiments comply with the current laws of the country in which they were performed.

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