Molecular Genetics and Genomics (2020) 295:121–133
https://doi.org/10.1007/s00438-019-01608-1

ORIGINAL ARTICLE

Identification of quantitative trait loci for kernel-related traits and the heterosis for these traits in maize (*Zea mays* L.)

Yinghong Liu[1] · Qiang Yi[2] · Xianbin Hou[3] · Yufeng Hu[2] · Yangping Li[2] · Guowu Yu[2] · Hanmei Liu[4] · Junjie Zhang[4] · Yubi Huang[2]

Received: 15 April 2019 / Accepted: 31 August 2019 / Published online: 11 September 2019

Abstract

Heterosis has been extensively applied for many traits during maize breeding, but there has been relatively little attention paid to the heterosis for kernel size. In this study, we evaluated a population of 301 recombinant inbred lines derived from a cross between 08-641 and YE478, as well as 298 hybrids from an immortalized F_2 (IF_2) population to detect quantitative trait loci (QTLs) for six kernel-related traits and the mid-parent heterosis (MPH) for these traits. A total of 100 QTLs, six pairs of loci with epistatic interactions, and five significant QTL × environment interactions were identified in both mapping populations. Seven QTLs accounted for over 10% of the phenotypic variation. Only four QTLs affected both the trait means and the MPH, suggesting the genetic mechanisms for kernel-related traits and the heterosis for kernel size are not completely independent. Moreover, more than half of the QTLs for each trait in the IF_2 population exhibited dominance, implying that dominance is more important than other genetic effects for the heterosis for kernel-related traits. Additionally, 20 QTL clusters comprising 46 QTLs were detected across ten chromosomes. Specific chromosomal regions (bins 2.03, 6.04–6.05, and 9.01–9.02) exhibited pleiotropy and congruency across diverse heterotic patterns in previous studies. These results may provide additional insights into the genetic basis for the MPH for kernel-related traits.

Keywords Heterosis · Kernel-related traits · Immortalized F_2 (IF_2) · RIL · QTL · Dominance

Introduction

The earliest application of heterosis was in maize, which is widely cultivated worldwide. The grain yield of maize hybrids increased by at least 15% over that of open-pollinated varieties (Duvick 1999). There are three hypotheses regarding the genetic basis for the better-parent performance phenomenon, namely dominance (Davenport 1908; Bruce 1910), overdominance (Shull 1908; East 1908), or epistasis (Powers 1944; Williams 1959). Many studies have focused on the complex genetic mechanisms underlying the heterosis for yield-related traits in maize (Stuber et al. 1992; Cockerham and Zeng 1996; Lu et al. 2003; Frascaroli et al. 2007; Garcia et al. 2008; Tang et al. 2010; Schön et al. 2010; Larièpe et al. 2012; Guo et al. 2014; Wang et al. 2016, 2018; Li et al. 2017; Samayoa et al. 2017). These studies involved the following heterotic patterns: 'Stiff Stalk Synthetic × Lancaster Sure Crop' (Stuber et al. 1992; Cockerham and Zeng 1996; Lu et al. 2003; Frascaroli et al. 2007; Garcia et al. 2008; Schön et al. 2010), 'synthetic × Flint' (Tang et al. 2010; Guo et al. 2014), 'Reid × Tangsipingtou' (Wang et al.

Communicated by Stefan Hohmann.

Yinghong Liu, Qiang Yi and Xianbin Hou have contributed equally to the work.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00438-019-01608-1) contains supplementary material, which is available to authorized users.

✉ Yubi Huang
yubihuang@sohu.com

[1] Maize Research Institute, Sichuan Agricultural University, Chengdu 611130, China

[2] State Key Laboratory of Crop Gene Exploration and Utilization in Southwest China and College of Agronomy, Sichuan Agricultural University, Chengdu 611130, China

[3] College of Agriculture and Food Engineering, Baise University, Baise 533000, Guangxi, China

[4] College of Life Science, Sichuan Agricultural University, Ya'an 625014, China

2016, 2018; Li et al. 2017), and 'American Dent × European Flint' (Samayoa et al. 2017). These investigations revealed that all of the genetic effects for diverse traits varied and played a different role depending on the heterotic patterns and genetic architecture of the population. However, there has been little research on the heterosis for kernel components in the heterotic pattern 'Reid × tem-tropic I (a synthetic population from the temperate × tropic I germplasm)', which has been widely applied in southwestern China.

Kernel length (KL), kernel width (KW), kernel thickness (KT), and hundred-kernel weight (HKW) are important traits influencing the contribution of individual ears to maize kernel yield. There are strong relationships among kernel-related traits, including length, width, and thickness (Li et al. 2009). Previous studies examining kernel-related traits involved $F_{2:3}$ families (Liu et al. 2014), recombinant inbred line (RIL) population (Yang et al. 2016; Liu et al. 2017; Zhang et al. 2017; Lan et al. 2018), immortalized F_2 (IF_2) population (Zhang et al. 2014, 2016), a four-way cross population (Chen et al. 2016a), and association panel (Zhang et al. 2017; Zhu et al. 2018). These studies identified many new and stable quantitative trait loci (QTLs) and single nucleotide polymorphisms (SNPs). For example, Liu et al. (2014) identified 12 stable major QTLs using $F_{2:3}$ families derived from the parental lines with highly significant differences in kernel-related traits. Additionally, Zhang et al. (2014, 2016) detected 54 unconditional loci and 97 conditional QTLs at different kernel development stages in an IF_2 population, which enabled the comprehensive characterization of the genetics mediating kernel development. Earlier investigations also applied the North Carolina Design III (Samayoa et al. 2017; Li et al. 2017), IF_2 population (Tang et al. 2010; Guo et al. 2014), and chromosome segment substitution line test populations (Wang et al. 2016, 2018) to identify the heterotic loci and the genetic basis for the heterosis for yield-related traits. However, there remains a lack of published research describing the heterosis for kernel-related traits. Frascaroli et al. (2007) highlighted the importance of heterotic loci showing dominance and epistasis for HKW in mapping populations derived from the heterotic pattern 'Reid × Lancaster' with an extensive design III. Tang et al. (2010) and Guo et al. (2014) stated that the heterosis for HKW was due to cumulative genetic effects (dominance and epistasis). Wang et al. (2018) detected 63 and 57 different QTLs regarding the heterosis for four kernel-related traits in two chromosome segment substitution line test populations derived from the Reid × TSPT (Tangsipingtou) cross, thereby providing the foundation for the fine-mapping of heterotic loci for kernel size. Notably, a large ratio of incongruent heterotic loci for various heterotic patterns indicated the complexity of the genetic mechanisms for the heterosis for kernel-related traits (Li et al. 2017; Wang et al. 2018). An analysis of kernel components and the heterosis for these traits provided new insights for elucidating the genetics underlying the heterosis for maize grain yield, especially for diverse heterotic patterns.

To date, numerous genes associated with maize grain-related traits have been identified, including *Opaque-2* (*O2*; Schmidt et al. 1987, 1992), *Sugary-1* (*Su1*; Pan and Nelson 1984), *Shrunken-2* (*Sh2*), and *Brittle-2* (*Bt2*; Dickinson and Preiss 1969). Several major QTLs, such as *qGW4.05* (Chen et al. 2016a, b), *qKW7* (Li et al. 2016), *qKL1.07* (Qin et al. 2016), and *qGW1.05* (Zhou et al. 2017), and *KNE4* (Zhan et al. 2018), were fine-mapped to narrow genomic regions. Notably, *ZmGS3* (Li et al. 2010a) and *ZmGW2-CHR4* (Li et al. 2010b), which are maize orthologs of rice genes, were identified by a homology-based cloning strategy. There are few major QTLs that have been cloned via map-based cloning, although a series of genes has been identified in the aforementioned previous studies.

In this study, we investigated the genetic basis of six kernel-related traits and the heterosis for these traits in two mapping populations. The RIL population comprised 301 lines derived from the cross between Ye478 and 08-641, whereas the IF_2 population was generated from the RIL population. The parental lines Ye478 and 08-641 belong to the improved Reid (PA) and tem-tropic I group, respectively. The 'PA × PB' heterotic pattern is widely applied in southwestern China. The objectives of this study were to (1) identify the QTLs for kernel-related traits in the RIL and IF_2 populations; (2) detect the heterotic loci for those traits; (3) identify the pleiotropic genomic regions and assess the genetic effects contributing to the heterosis in the present heterotic pattern; and (4) provide valuable information for characterizing the genetic basis of the heterosis for these traits.

Materials and methods

Plant materials and field experiments

The single-seed descent method was performed with the Ye478 × 08-641 hybrid to produce a set of 301 RILs (Liu et al. 2016). The 320 RILs before filtering the RILs with high heterozygosity were randomly divided into two groups of 160 RILs as described by Hua et al. (2002) and Tang et al. (2010). Paired crosses between the lines of both groups were performed according to the one-to-one method, thereby producing 160 F_1 crosses. After repeating the procedure twice, we ultimately generated 320 crosses. We discarded the RILs with heterozygosity greater than 20% and their corresponding crosses, and an IF_2 population of 298 F_1 crosses was generated.

The RIL population, the parental lines, and the F_1 hybrid were planted in four environments: three at the

Xishuangbanna Maize Breeding Base of the Maize Research Institute of Sichuan Agricultural University, Jinghong (JH; 21°95′N, 100°76′E), in Yunnan province, China in March 2014 (JH2014), March 2015 (JH2015), and March 2016 (JH2016) as well as one at the Modern Agriculture Research and Development Center of Sichuan Agricultural University, Chongzhou (CZ; 30°33′N, 103°38′E), in Sichuan province, China on April 2016 (CZ2016). The IF_2 population was evaluated under three environments (i.e., 2015JH, 2016JH, and 2016CZ). The field experiments were performed following a randomized complete block design with two repetitions. Each plot consisted of 3-m-long rows, each consisting of 14 plants, with 0.80 m separating rows. Finally, each trial was evaluated with a plant density of 57,000 plants per ha. In each environment, both mapping populations were planted adjacent and occupied a square and uniform parcel. The trials included the application of fertilizers, fungicides, and pesticides based on normal agronomic practices. Irrigation and manual weeding were applied as needed for the intensive yield of each season.

Phenotypic measurements and analysis

All plants in each plot were harvested and after measuring the total ear weight, ten well-pollinated ears in the middle of each row were randomly chosen for additional kernel evaluations. Six kernel-related traits were assessed as previously described (Liu et al. 2014). Specifically, the ear length without kernels (WKEL, cm) was calculated as the average of ten measurements of the length from the kernels at the tip of the ear to the ear tip without kernels. The HKW (in g) was calculated as the average of three replications, with the equation, HKW × (1 − moisture %)/(1 − 14%). A hundred kernels were randomly chosen from the bulked kernels and weighed with an electronic balance. The KL (in mm) was calculated as the average of three measurements of ten consecutive kernels selected from the bulked kernels from the middle of the ears. The KW (in mm) was calculated as the average of three measurements of ten consecutive kernels selected from the bulked kernels from the middle of the ears, whereas KT (in mm) was calculated as the average of three measurements of five consecutive kernels from the middle of the ears with electronic digital calipers. The volume weight (VW; in g/L) (i.e., kernel weight per unit volume) was calculated as the average of two replicated measurements of the bulked kernels with the Shanghai Qingpu LSD-G volume weight tester according to the SAC method GB1353-2009 (SAC 2009). The bulked kernels were dried under natural conditions for 15 days before the measurement (moisture content less than 14%). The average data for the phenotypic traits of the 301 RILs and the IF_2 population across all environments were analyzed for the distribution of the phenotypic traits and Pearson's correlations with PROC CORR from the statistical software package SPSS 17.0 (http://www.spss.com). Combined analyses of variance for each trait of the RIL and IF_2 populations were estimated with the GLM procedure of SPSS 17.0, with genotypes as fixed effects and replications and environments as random effects. Broad-sense heritability (H^2) for the two mapping populations was calculated on an entry mean basis as described by Hallauer and Miranda (1988): $h^2 = \sigma_g^2 \Big/ \left(\sigma_g^2 + \frac{\sigma_{ge}^2}{n} + \frac{\sigma^2}{nb}\right)$, where σ_g^2 denotes the genetic variance, σ_{ge}^2 is the genotype × environment interaction variance, σ^2 represents the residual error variance, b refers to the number of replications, and n is the number of environments. The 90% confidence interval of heritability $\left(h^2\right)$ was estimated as described by Knapp et al. (1985). The phenotypic traits of both mapping populations were analyzed with two mixed models fitted by the restricted maximum likelihood approach:

$$Y_{mk} = \mu + G_m + R_k + \varepsilon_{mk} \quad (1)$$

$$Y_{mik} = \mu + G_m + GE_{mi} + E_i + R_k + \varepsilon_{mik} \quad (2)$$

where Y_{mk} denotes the phenotypic value of genotype m in replication k, Y_{mik} denotes the phenotypic value of genotype m in environment i and replication k, μ represents the overall mean of the RIL population or the IF_2 population, G_m is the random effect of genotype m, GE_{mi} is the random effect of the interaction between genotype m and environment i, E_i is the random effect of environment i, R_k is the random effect of replication k, and ε_{mk} and ε_{mik} represent the random experimental errors. The best linear unbiased predictions (BLUPs) for the single-environment analysis and combined analysis across all environments were performed with Eqs. (1) and (2), respectively. The adjusted means were used for the subsequent QTL mapping. The analyses were conducted with the R program for statistical computing (R Development Core 2010; lme4 package, Bates et al. 2015). The mid-parent heterosis (MPH) was calculated as follows: $MPH_{ij} = F_{ij} - \left(P_i + P_j\right)/2$ (Hua et al. 2003), where F_{ij} is the BLUP value of the F_{ij} hybrid (F_1 cross) from the IF_2 population and $\left(P_i + P_j\right)/2$ is the average BLUP values for the corresponding parents (P_i and P_j from the RIL population). The genotypic dataset for the MPH depends on the dominance and epistatic effects, but epistatic effects were not included and an additive-dominance model has been assumed for the IF_2 population (Tang et al. 2010; Guo et al. 2014).

Molecular linkage map construction

Genomic DNA was extracted from the juvenile leaves of 7-day-old seedlings of the RIL population and the parental inbreds (each bulk comprising ten plants per line) according to a modified CTAB method (Chen and Ronald 1999). An oligonucleotide pool assay involving 3072 SNPs was

developed by the National Maize Improvement Center of China based on Illumina GoldenGate technology. The markers were used for genotyping the RIL population and the corresponding parents. After screening for heterozygous data points (< 20%), missing data points (< 20%), and segregation distortion, 683 SNPs that were distributed across all ten maize chromosomes were used to construct a linkage map, with a total length of 1786.1 cm and an average interval of 2.61 cm. The genetic map for the RIL population was generated with MapDisto 1.7.5 (http://mapdisto.free.fr/DL/) (Mathias 2012). The Kosambi mapping function was used for converting recombination frequencies to genetic distances (Kosambi 1943). Regarding the IF_2 population, the genotyping data of every cross were deduced from the marker data of the corresponding RIL parents. The genetic linkage map of the RIL population was also used for the IF_2 population and the MPH dataset (Hua et al. 2002; Tang et al. 2010).

QTL mapping

For the RIL population, the IF_2 population, and the MPH dataset, QTLs for each trait were identified with the inclusive composite interval mapping (ICIM) (Li et al. 2007, 2008) of the QTL ICIMapping software (Meng et al. 2015). We used the ICIM-ADD method, with the *p* value set at 0.001 for entering variables in the stepwise regression of residual phenotypes on marker variables (PIN), whereas the *p* value was set at 0.002 for removing variables to detect QTLs. An empirical threshold LOD score was used for declaring significant QTLs based on a 1000-permutations test, with a type I error rate of 0.05 (Doerge and Churchill 1996) and a step size of 1 cm. Single marker analysis (SMA) to detect loci with the same empirical threshold LOD was performed for the RIL and IF_2 populations. The degree of dominance was defined based on the dominance effect to additive effect ratio (|D/A|) for each QTL (Guo et al. 2014). A |D/A| > 1.26 reflected the overdominance of a locus, otherwise, the locus was considered as dominance type (Falconer and Mackay 1996). The QTLs were nominated as follows: 'q' + 'the abbreviation of the trait' + 'chromosome' + '−' + 'physical order or omission within the chromosome'. Overlapping or adjacent QTLs for different traits were regarded as a QTL cluster. The QTL and QTL density across all chromosomes was plotted with the R program (R Development Core 2010). In addition, the epistatic interaction (EPI) and the QTL × environment interaction (QEI) were analyzed according to the mixed-model-based composite interval mapping (MCIM) using the QTLNetwork program 2.1, (Wang et al. 1999; Yang et al. 2007, 2008). An additive by additive (AA) interaction in the RIL population was detected via combined analysis across all environments. For the IF_2 population, the following four types of digenic effects were detected: AA interaction, additive by dominant (AD) interaction, dominant by additive (DA) interaction, and dominant by dominant (DD) interaction via combined analysis across all environments. We also detected these four types of EPIs in the MPH dataset based on the average data across environments. The QEI effects were analyzed in the RIL and IF_2 populations via a combined analysis across all environments. The QTLs for each trait were identified along with the full QTL model containing significant additive, dominance, and epistatic effects, as well as their interactions with environments (testing window and filtration window size of 10 cm and a walking speed of 2 cm). The critical *F* value for selecting candidate intervals was determined by 1000-permutation tests with $p < 0.05$ as the experimental-wise significance level. Bonferroni correction was used to compute the comparison-wise significance threshold, assuming an experiment-wise error rate < 0.05 (Rice 1989).

Results

Phenotypic variations of kernel-related traits and the heterosis for these traits

The parental lines Ye478 and 08-641 significantly differed regarding KL, KW, and HKW (Fig. 1a). Considerable phenotypic variations for all traits were also observed in the RIL and IF_2 populations (Fig. 1b). The mean KL and KW were significantly higher for the F_1 hybrid than for 08-641, and the average WKEL was significantly higher for the F_1 hybrid than for either parent. The average MPH for the KT, KL, KW, and HKW of the F_1 hybrid were − 2.93 mm, 1.21 mm, 1.44 mm, and 1.68 g, respectively (Table S1). Similarly, the average MPH varied from − 13.5% for KT to 12.2% for KL in the IF_2 population. The average WKEL for the F_1 hybrid was 0.83 cm (246.2%), whereas the mean MPH of WKEL in the IF_2 population was − 0.56 cm (− 23.3%). These results indicated the mean KL, KW, VW, and HKW were higher for the hybrid than for the parental lines, but the WKEL and KT exhibited the opposite trend.

Highly significant genotype, environment, genotype × environment interaction effects were detected for all traits ($p < 0.01$; Table 1). Similar and moderate broad-sense heritability (h^2) was observed for KT, KL, KW, HKW, and VW across the RIL and IF_2 populations, ranging from 60.4% for KT to 77.0% for KW in the RIL population. The heritability for WKEL was greater in the RIL population (74.5%) than in the IF_2 population (64%). There were low-to-moderate significant correlations among kernel-related traits and the MPH for these traits (Table S2). Specifically, HKW was significantly positively correlated with WKEL, KT, KL, and KW across the RIL and IF_2 populations. There were also weakly significant correlations between VW and KL, VW

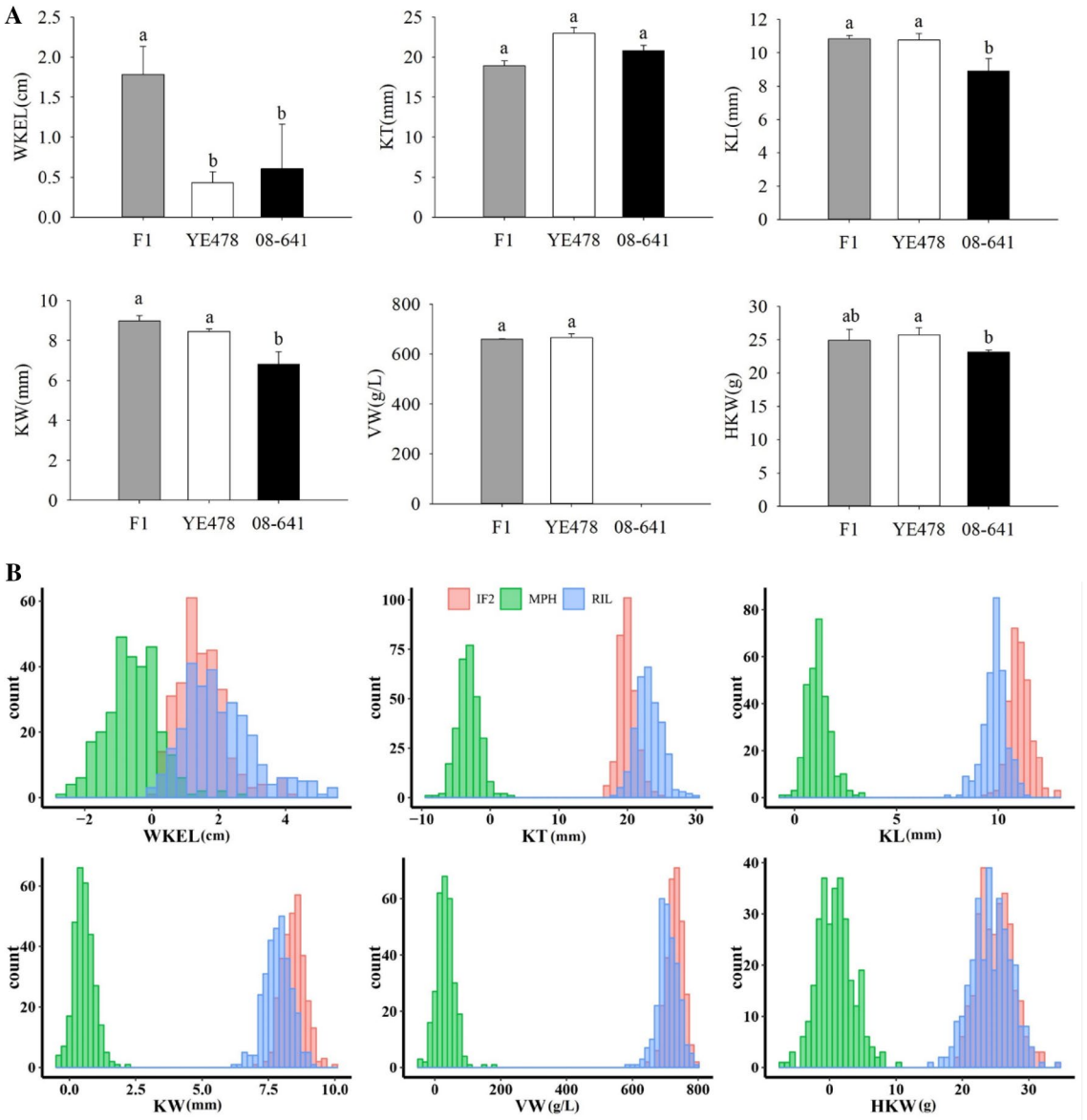

Fig. 1 Performance of six kernel-related traits for the parental lines and the hybrid F_1 along with both mapping populations and the MPH dataset. **a** Comparison of means for six kernel-related traits among inbred Ye478, 08-641 and the F_1 hybrid. The Duncan multiple range test was used for the comparison of means. Genotypes with lowercase letters were significantly different at the 0.05 probability level. Means with same letters are not significantly different. The data are shown as mean ± SD (standard deviation). **b** The histogram for kernel-related traits and mid-parent heterosis (MPH) for those traits in the RIL and IF_2 population. *WKEL* ear length without kernels, *KT* kernel thickness, *KL* kernel length, *KW* kernel width, *VW* volume weight, *HKW* hundred-kernel weight

and KW, KW and KT, and KW and KL across both mapping populations. The MPH for HKW was significantly correlated with the MPH for KT, KL, and KW ($p < 0.01$). Additionally, the MPH for KL was significantly negatively correlated with WKEL and KT but was significantly positively correlated with KW ($r = 0.54$, $p < 0.01$). Moreover, a significantly positive correlation was detected between WKEL and KT across the mapping populations, as well as between the MPH for WKEL and KT ($p < 0.01$). These results revealed the strong correlations among yield components.

Identification of QTLs for kernel-related traits and the heterosis for these traits

The main features of 100 putative QTLs for the six traits investigated in this study are summarized in Table S3. More than half of the QTLs were simultaneously identified via single environment analysis and combined analysis across all environments (Table S4). Each QTL accounted for 0.40–25.89% of the phenotypic variation, with the contribution of more than half of these QTLs less than 5% (Fig. 2).

Table 1 Mean squares from the combined analyses of variance and heritabilities on a family mean for kernel-related traits in the RILs and IF_2 population under three environments

Trait	Population	Gen	Env	Rep (Env)	Gen × Env	h^2 (%)	CI (%)
WKEL	RIL	6.02**	379.36**	4.78*	1.54**	74.5	69.7–78.3
	IF_2	2.90**	324.29**	8.04**	1.04**	64.0	57.3–69.5
KT	RIL	17.94**	221.03**	0.76	7.03**	60.8	53.6–66.8
	IF_2	8.50**	313.15**	1.87	3.22**	62.1	55.0–67.9
KL	RIL	1.60**	593.36**	1.47*	0.50**	68.9	63.2–73.6
	IF_2	1.44**	604.94**	14.41**	0.46**	68.1	62.2–73.0
KW	RIL	1.22**	206.36**	0.06	0.28**	77.0	72.7–80.5
	IF_2	0.99**	288.82**	11.58**	0.23*	76.9	72.6–80.5
VW	RIL	2997.5**	53656.70**	4220.24**	994.75**	66.8	59.1–73.2
	IF_2	2882.10**	820810.32**	2.91	943.45**	67.3	61.1–72.3
HKW	RIL	43.3**	2317.04**	0.16	11.03**	74.5	70.0–78.2
	IF_2	35.24**	12599.91**	20.80**	9.22**	73.8	68.9–77.9

WKEL ear length without kernels, *KT* kernel thickness, *KL* kernel length, *KW* kernel width, *VW* volume weight, *HKW* hundred-kernel weight, *RIL* recombinant inbred lines, *IF_2* immortalized F_2, *Gen* genotype, *Env* environment, *Rep*(*Env*) replication nested within the environment, *Gen*×*Env* genotype×environment interaction, h^2 broad-sense heritability, *CI* the confidence interval of broad-sense heritability

*Significant at $p < 0.05$

**Significant at $p < 0.01$

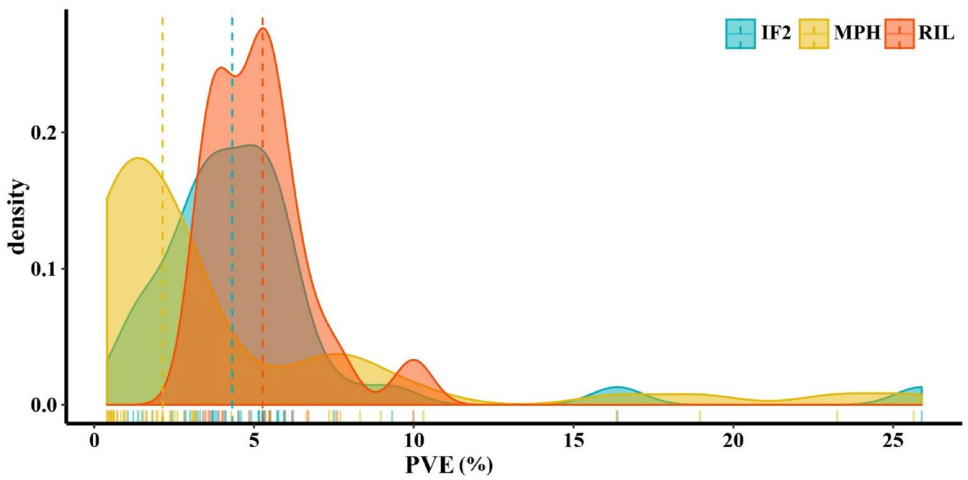

Fig. 2 Frequency distribution of QTL with PVE (phenotypic variance explained by each QTL) for six kernel-related traits and the mid-parent heterosis (MPH) level in the RILs and IF_2 population. The vertical and dashed lines indicate the median values

The number of QTLs varied from two for KT and VW in the RIL population to 11 for KW in the IF_2 population. Additionally, two QTLs were simultaneously detected across the RIL and IF_2 populations, and two QTLs in the RIL population also influenced the MPH. Only two heterotic loci were co-mapped in the IF_2 population. Moreover, 16, 34, and 44 QTLs were specific for the RIL population, IF_2 population, and the MPH dataset, respectively.

We identified 18 QTLs for WKEL, five QTLs in the RIL population, four QTLs in the IF_2 population, and ten QTLs for MPH (Table S3; Fig. 3). These QTLs were distributed across all chromosomes, except for chromosomes 4 and 5. The total contribution of all QTLs was similar in both mapping populations and the MPH dataset, and individual QTLs explained between 1.48% (for MPH) and 16.37% (for the IF_2 population) of the phenotypic variation [i.e., phenotypic variation explained (PVE)]. The environmentally stable QTL *qWKEL3-1*, which was specific for the RIL population, had a high PVE of 9.99% and an additive effect of − 0.24 cm. At the *qWKEL2-2* locus, which was present across the RIL and IF_2 populations, the Ye478 allele decreased the WKEL. In addition, most of the heterotic loci with minor effects had negative dominance effects.

For KT, two QTL in the RIL population, three QTL in the IF_2 population, and eight heterotic loci were identified across all chromosomes, except for chromosomes 9 and 10. Each QTL explained a range of 2.4–10.3%. The total PVE was considerably higher for MPH than for either mapping populations. At bin 8.06, the IF_2-specific *qKT8-1* and the heterotic locus *qKT8-1* were detected under two single environments. The *qKT8-1* locus for MPH (bin 2.08) had

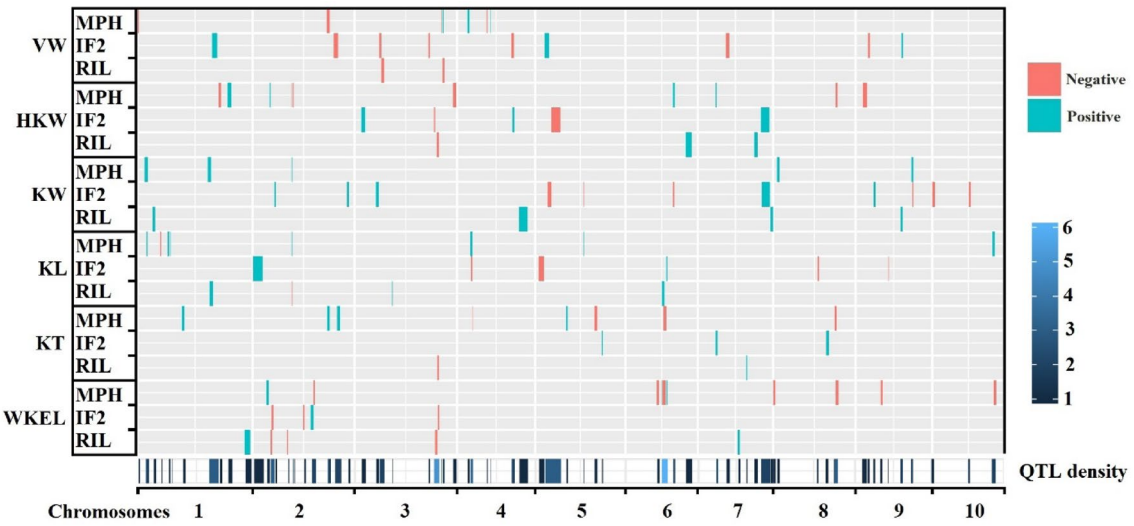

Fig. 3 QTL distribution for six kernel-related traits and heterosis for those traits in the RIL and IF_2 populations. The red rectangles indicate QTLs with negative additive effects (or dominant effects for mid-parent heterosis (MPH)), while the blue rectangles represent positive additive effects (or dominant effects for MPH). The width of the rectangle indicates the confidence interval of QTL. The heatmap of QTL density across ten chromosomes was indicated at the bottom of the figure and below the *X*-axis. *WKEL* ear length without kernels, *KT* kernel thickness, *KL* kernel length, *KW* kernel width, *VW* volume weight, *HKW* hundred-kernel weight

the highest PVE (10.3%). No QTLs were identified in both populations as well as in the MPH dataset.

For KL, we identified 16 QTLs across all chromosomes, except for chromosome 7, as well as four QTLs in the RIL population, six QTLs in the IF_2 population, and eight QTLs for MPH. Of these QTLs, nine had a PVE of less than 2%. The IF_2-specific *qKL9* harboring the favorable allele from 08-641, had the highest PVE (25.86%). The QTL *qKL2-2* for MPH was also co-located in the RIL population, but not in the IF_2 population. Another locus in the IF_2 population, *qKL4*, which exhibited an overdominance effect, had a similar dominance effect on MPH, and the favorable allele was contributed by 08-641.

For KW, we detected four QTLs in the RIL population, 11 QTLs in the IF_2 population, and five heterotic loci across all chromosomes. The total PVE in the IF_2 population was much higher than in the RIL population or the MPH dataset. Individual QTLs had a PVE between 0.59 and 16.35% for MPH. The environmentally stable QTL *qKW3* had an additive effect of 0.13 mm and a PVE of 7.52% in the IF_2 population. Notably, *qKW9-3* exhibited an overdominance effect in the IF_2 population, and also affected the MPH for KW.

Sixteen QTLs were identified for VW, with two QTLs in the RIL population, nine QTLs in the IF_2 population, and seven QTLs for MPH. The total contribution of all QTLs for VW was similar to that for KW in both populations and the MPH dataset. The QTL *qVW5* in the IF_2 population, which was detected in two single-environment analyses, had the highest PVE (9.32%) and harbored the favorable allele from Ye478. The QTL *qVW3-1* at bin 3.04 was simultaneously detected in the RIL and IF_2 populations, and the favorable allele was contributed by 08-641. The heterotic locus *qVW3-4* exhibited a high dominance effect and was also identified in the RIL population.

Eighteen QTLs were detected for HKW, with three QTLs in the RIL population, five QTLs in the IF_2 population, and ten QTLs for MPH. These QTLs were distributed across all chromosomes, except for chromosome 10. Individual QTLs had a PVE of 0.40–23.24% for MPH. Five of the alleles derived from Ye478 in both populations were associated with increases in HKW. Most of these heterotic loci explained less than 1% of the phenotypic variation. There was no QTL congruency among both populations and the MPH dataset.

Analysis of the degree of dominance on heterosis

The |D/A| ratio for each locus (Falconer and Mackay 1996; Guo et al. 2014) calculated based on SMA and ICIM (Fig. 4) was used to assess the relative importance of the degree of dominance on the heterosis for kernel-related traits. In the IF_2 population, more than 70% of the genomic markers for KW exhibited dominance ($|D/A| < 1.26$), as did over 60% of the genomic markers for VW and over 55% of the genomic markers for WKEL, KT, KL, and HKW. Regarding the degree of dominance estimated for the RIL and IF_2 populations, more than 60% of the genomic markers for WKEL and HKW, more than 50% of the genomic markers for KW, and more than 39% of the genomic markers for KT, KL, and VW exhibited dominance. The SMA results indicated that more

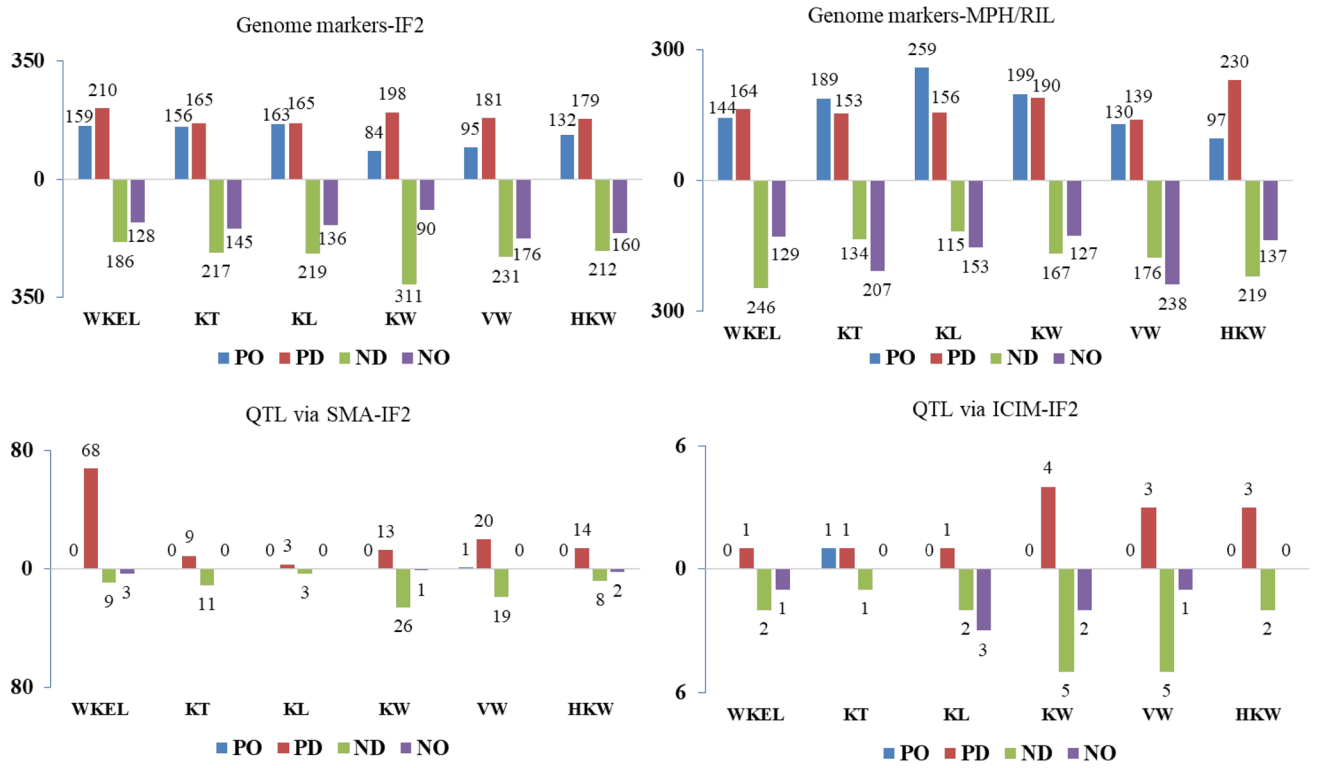

Fig. 4 Distribution of markers in the whole genome and significant loci for all six kernel-related traits exhibiting dominance and overdominance effect. The degree of dominance was defined as a ratio of the dominance effect to additive effect (D/A) for each marker and QTL (Guo et al. 2014). For the IF_2 population, the ratio D/A was calculated by a ratio of dominance effects to additive effects for genomic markers and QTL. The ratio D/A of genomic markers (MPH/RIL) was estimated by the ratio of additive effects from the RIL population to dominance effects from the MPH dataset. *SMA* single-marker analysis, *ICIM* inclusive composite interval mapping, *PO* positive overdominance, *PD* positive dominance, *ND* negative dominance, *NO* negative overdominance, *WKEL* ear length without kernels, *KT* kernel thickness, *KL* kernel length, *KW* kernel width, *VW* volume weight, *HKW* hundred-kernel weight

than 90% of the significant markers for all traits exhibited dominance in the IF_2 population. Nearly 50% of the QTLs for KL exhibited dominance, as did 66.7% of the QTLs for KT, 75% of the QTLs for WKEL, over 80% of the QTLs for KW and VW, and 100% of the QTLs for HKW. These findings suggested that the loci exhibiting dominance contributed to the heterosis for kernel-related traits, especially for WKEL, HKW, and KW. Additionally, a weakly significant correlation was detected between total heterozygosity and VW variations. Furthermore, total heterozygosity was weakly significantly correlated with the MPH for KT, KL, and VW (Table S5).

Analysis of the epistatic interaction and the QTL × environment interaction

In the present study, we analyzed digenic interactions in both populations and the MPH dataset (Table S6). Six pairs of loci with low heritability ($h^2 < 2\%$) were associated with EPIs, with three pairs for HKW in the RIL population and the remaining pairs for WKEL, KT, and KW in the IF_2 population. Notably, epistasis was not detected for MPH. We also analyzed the QEIs (Table S7). Only five loci with low contributions interacted with environments, with two loci in the RIL population and three loci in the IF_2 population. Many of these loci exhibiting EPIs and QEIs were co-located with QTLs for the trait means. For example, the major QTL for WKEL, *qWKEL2-4*, interacted with the locus on chromosome 7 exhibiting AA and DD interactions, and also interacted with the 2016JH environment.

QTL pleiotropy

This study included an investigation of QTL pleiotropy. A total of 20 QTL clusters comprising 46 QTLs were detected across all chromosomes (Table 2). Some of these QTLs were localized to the genomic regions related to various kernel-related traits and the heterosis for these traits, suggesting that these loci affected the phenotypic variation and MPH of various traits. Pleiotropic effects were detected for half of these clusters. For example, QC1-2 influenced both KL and the MPH for KW, whereas QC6-3 affected KW and the MPH for HKW. We also detected five clusters that affected various trait means,

Table 2 QTL clusters for kernel-related traits and mid-parent heterosis detected in the RILs and IF_2 population

QTL Cluster[a]	Bin[b]	Interval (cm)	Flanking marker	No. of QTL	Traits influenced	References
QC1-1	1.02	14.5–21.5	PZE-101026314/PZE-101027182	2	KL(M), KW(M)	
QC1-2	1.07	148.5–159.5	SYN376/SYN2411	2	KL(R), KW(M)	
QC2-1	2.03	29.5–38.5	PZE-102037260/PZE-102047187	2	WKEL(M), HKW(M)	Wang et al. (2018)
QC2-2	2.03–2.04	40.5–49.5	PZE-102047571/PZE-102056295	2	WKEL(R, I), HKW(I)	Chen et al. (2017)
QC2-4	2.05	83.5–84.5	PZE-102094429/PZE-102097841	3	KL(R, M), KW(M), HKW(M)	Chen et al. (2017)
QC2-5	2.08	157.5–163.5	SYN7501/PZE-102172290	2	KT(M), VW(M)	Zhang et al. (2016), Chen et al. (2017)
QC2-6	2.08–2.09	172.5–185.5	PZE-102176967/SYN7209	2	VW(I), KT(M)	
QC3	3.08	174.5–179.5	PZE-103161091/PZE-103163529	3	WKEL(I), KT(R), HKW(R)	
QC4	4.07	115.5–121.5	PZE-104097453/PZE-104099837	2	VW(I), HKW(I)	
QC5-1	5.00–5.03	26.5–53.5	PZE-105006095/PZE-105032498	3	KW(I), HKW(I), VW(I)	Chen et al. (2017)
QC5-2	5.04	102.5–103.5	PZE-105102631/SYN38374	2	KL(M), KW(I)	Li et al. (2010a, b)
QC6-1	6.04–6.05	77.5–86.5	SYN7240/PZE-106068277	3	WKEL(M), KL(R), KW(M)	Chen et al. (2017), Wang et al. (2018)
QC6-2	6.05	86.5–88.5	PZE-106069732/SYN38352	2	WKEL(M), KL(R)	
QC6-3	6.05	100.5–104.5	PZE-106083557/PZE-106083588	2	KW(I), HKW(M)	
QC7-1	7.01	35.5–41.5	SYN19841/PZE-107012088	3	KT(I), HKW(M)	
QC7-2	7.04–7.05	133.5–152.5	PZE-107121485/PZE-107130438	2	KW(I), HKW(I)	Guo et al. (2014), Wang et al. (2018)
QC8	8.06	130.5–138.5	PZE-108110152/SYN30185	3	KT(M), HKW(M), WKEL(M)	Zhang et al. (2016), Chen et al. (2017)
QC9-1	9.01–9.02	15.5–30.5	PZE-109008839/SYN6084	2	HKW(M), VW(I)	Wang et al. (2018)
QC9-2	9.03–9.04	95.5–100.5	PZE-109057210/SYN37647	2	KW(R), VW(I)	Chen et al. (2017)
QC10	10.07	127.5–135.5	PZE-110104601/SYN22564	2	KL(M), WKEL(M)	Zhang et al. (2016), Chen et al. (2017)

WKEL ear length without kernels, *KT* kernel thickness, *KL* kernel length, *KW* kernel width, *VW* volume weight, *HKW* hundred-kernel weight, *L* RIL population, *I* the IF_2 population, *M* mid-parent heterosis

[a]The nomination of QTL cluster is made of three parts. The first part is a "QC" standing for the abbreviation of QTL cluster. The second part is a number standing for chromosome and the third part is a number standing for the physical order

[b]The genetic region included the position of QTL. (http://www.maizegdb.org/)

but not the MPH; three of these clusters were specific for the IF_2 population and two were identified in both mapping populations. The QC5-1 cluster at bin 5.00–5.03 had a pleiotropic effect on KW, HKW, and VW in the IF_2 population. Additionally, five clusters contained overlapping QTLs specific for the MPH for various traits. The QC1-1 cluster, which was in a 7 cm region at bin 1.02, affected the MPH for KL and KW. Moreover, QC10 at bin 10.07 contained three QTLs for the MPH for WKEL and KL, which were mapped to the same position.

Discussion

QTL congruency for kernel-related traits

Maize kernel yield is influenced by a long development period involving many complex factors. Kernel size and weight are secondary traits but are very important for the final kernel yield (Li et al. 2009; Liu et al. 2014). Considering kernel size as a whole, there are also strong

relationships among the kernel components investigated in our study (Liu et al. 2014, 2017; Lan et al. 2018). Compared with the corresponding mid-parent values, the KT was lower, whereas KL, KW, and HKW were higher in the hybrids. Although we observed moderate-to-high heritability for the kernel-related traits, many of the detected QTLs were still specific for the populations and environments. Recent studies identified numerous QTLs or SNPs for kernel-related traits in diverse genetic populations (Liu et al. 2014, 2017; Zhang et al. 2014, 2016, 2017; Chen et al. 2016a, b, 2017; Yang et al. 2016; Lan et al. 2018; Zhu et al. 2018). Some QTLs identified in the current study were also detected in the reported studies. Nine QTLs (*qKT8-1*, *qKT5-3*, *qKL1-5*, *qKL3*, *qKW2-1*, *qKW2-3*, *qKW3*, *qKW9-1*, *qKW9-2*, *qKW10-2*, and *qVW3-2*) overlapped with the meta-QTLs affecting kernel-related traits summarized by Chen et al. (2017). Of these QTLs, Zhang et al. (2016) revealed that *qKT5-3* for KT overlapped with *conqKV5* for kernel volume between 190 and 191 Mbp on chromosome 5 in an analysis during developmental stage DAP43|DAP36 (i.e., when kernel volume at 43 days after pollination was conditioned on kernel volume at 36 days after pollination). Three clusters (QC2-2, QC5-1, and QC9-2) were co-localized with the meta-QTLs for grain yield, ear-related traits, and kernel-related traits identified by Chen et al. (2017). The QTLs *qVW7* (for VW) and *qKV7* (for kernel volume), which overlapped between 103 and 109 Mbp on chromosome 7 at 50 days after pollination, were also detected in an IF_2 population by Zhang et al. (2016). In addition, *qKT3* and *qHKW3-3* at bin 3.08 were mapped in the same interval and contained the significant SNP chr3.S_212832592 associated with KT and HKW in 10 RIL populations reported by Liu et al. (2017). Interestingly, *qKL2-1* for KL reportedly contains an important linkage disequilibrium block affecting kernel shape in previously analyzed RILs and in an association panel (Zhang et al. 2017). Additionally, *qVW3-4*, which influences the VW mean and the MPH for VW, is located close to the *Shrunken-2* gene (*sh2*; Dickinson and Preiss 1969) at position 216.4 Mb on chromosome 3; this gene helps control starch levels in the maize endosperm. Three QTLs, *qKT1*, *qKW4*, and *qKW5-2*, included or were close to three maize orthologs of cloned rice genes, *ZmGS3* (Li et al. 2010a), *ZmGW2-CHR4*, and *ZmGW2-CHR5* (Li et al. 2010b), respectively. Notably, *qKW5-2* for KW and *qKL5-2* for the MPH for KL were mapped in the same position. These findings suggested that the underlying loci for the final kernel yield may affect kernel development after pollination. Moreover, there may be a common genetic mechanism of kernel-related traits or such causal relationships across different backgrounds. Furthermore, interval co-mapping with known genes may be useful for clarifying the specific phenotypes associated with these genes. Future studies will need to verify these potential relationships.

Dominance effects account for the MPH for kernel-related traits

Dominance (Davenport 1908; Bruce 1910), overdominance (Shull 1908; East 1908), or epistasis (Powers 1944; Williams 1959) are genetic effects that have been used to explain the genetic mechanism underlying heterosis. In the current study, dominance was exhibited by over half of the QTLs for kernel-related traits. The total PVE of the heterotic loci for KT, KL, and HKW was more than 39%, and higher than that in the RIL and IF_2 populations. However, the total PVE of the QTLs for KW and VW in the IF_2 population was more than 43%, and much higher than that in the RIL population and that for the MPH for KW and VW. These results suggested that the low degree of dominance contributed to the heterosis for kernel-related traits, and are consistent with the phenotypic differences between the F_1 and parental lines. However, no EPIs were detected for MPH. Thus, the genetic basis for kernel-related traits and the heterosis for these traits with low heritability (data not shown) may be mainly explained by single-locus QTLs. However, statistical and experimental errors may have substantially influenced our ability to detect EPIs for the heterosis for kernel-related traits. Compared with the results of our study, an investigation by Frascaroli et al. (2007) revealed a lower total PVE for the heterotic loci and EPIs for HKW following the Reid × Lancaster hybridization. Tang et al. (2010) proposed that the dominance effects of heterotic loci at the single-locus level as well as the AA interactions are important genetic factors related to the heterosis for HKW. Additionally, Guo et al. (2014) concluded that the cumulative dominance effects, including dominance, overdominance, and epistasis, were responsible for most of the heterosis for HKW. Therefore, the heterotic loci for kernel-related traits at the single-locus level appear to be important for the final kernel yield. These findings may also be relevant for future attempts at fine-mapping the heterotic loci for kernel-related traits. Moreover, the weakly significant correlations between total heterozygosity and the MPH for KT, KL, and VW suggest that the overall heterozygosity minimally affects kernel-related traits and heterosis (Hua et al. 2003), which is inconsistent with the results of studies by Frascaroli et al. (2007) and Larièpe et al. (2012). Other earlier investigations confirmed that the detection of QTLs is biased by dominance, epistasis, and the linkage disequilibrium between QTLs (Melchinger et al. 2007; Schön et al. 2010; Tang et al. 2010; Guo et al. 2014). In the current study, only six QTLs were identified in the RIL and IF_2 populations as well as in the MPH dataset. Additionally, 94% of the QTLs were specific for the MPH or the populations, implying heterosis

and performance per se are controlled by different genetic mechanisms (Tang et al. 2010; Wei et al. 2016; Wang et al. 2018). A more thorough examination of the type of gene action revealed a low degree of dominance for most of QTLs in the IF_2 population. For example, *qKL4* and *qKW9-3* in the IF_2 population, with $|D/A| > 3$, were also identified in the MPH dataset, but not in the RIL population, indicating that loci with large dominance effects result in the biased detection of additive effects. The QTL *qWKEL2-4*, which exhibited an EPI and QEI, was only identified in the IF_2 population, suggesting that EPIs and QEIs may influence the detection of QTLs. Inconsistencies in the QTLs may be partly due to the types of gene action, and there may be identical genetic models of action for the MPH and hybrid performance per se (Guo et al. 2014).

Cumulative influence of kernel-related traits on the heterosis for yield

Similar to a previous study (Wang et al. 2018), we revealed low-to-moderate significant correlations among the MPH for kernel-related traits, including *r*(KL, KT), *r*(KL, KW), *r*(HKW, KL), and *r*(HKW, KW). As expected, ten QTL clusters were associated with the overlapping relationships among the heterosis for diverse traits. However, few consistent QTLs were observed to simultaneously affect the trait means and the MPH. On the basis of significant correlations and pleiotropic regions, the heterosis for kernel-related traits is not completely trait-specific (Flint-Garcia et al. 2009). We also noticed that the current F_1 hybrid between Ye478 and 08-641 exhibited high heterosis for kernel yield, with a considerably higher kernel weight per ear and kernel number per row when compared with the parental lines (data not shown). Nevertheless, there were no significant differences in the KT, KL, KW, VW, and HKW between the F_1 hybrid and the inbred parent Ye478. It seems that the high heterosis for yield may be due to the cumulative influence of the heterosis for kernel size (Flint-Garcia et al. 2009), which may help to explain why the ratio of the loci exhibiting overdominance for ear weight per ear, kernel weight per ear, and kernel number per row was greater than that for the kernel-related traits.

QTL pleiotropy influenced the kernel-related traits and heterosis

Many studies have addressed the QTL pleiotropy for yield-related traits and for the MPH for these traits (Frascaroli et al. 2007; Chen et al. 2017; Li et al. 2017; Zhang et al. 2017; Wang et al. 2018). In this study, we detected 20 QTL clusters, some of which were mapped to the same positions of clusters derived from other heterotic patterns (Wang et al. 2018). However, few investigations have focused on the heterosis for kernel-related traits. The QC2-1 region affected the MPH for WKEL and HKW and overlapped with the pleiotropic region for the MPH for KT, KL, KW, and HKW between 18 and 24 Mbp on chromosome 2 in the progeny of the crosses from Reid × TSPT (Wang et al. 2018). The QC6-1 region for KL and the MPH for WKEL and KW overlapped with *MQTL-42*, which controls ear-related traits and kernel-related traits (Chen et al. 2017), and is co-localized with *hKL6a* for the heterosis for KL (Wang et al. 2018). At bin 9.01-9.02, QC9-1 was observed to affect the VW and MPH for HKW, and overlapped with the heterotic loci for KT and HKW between 13 and 16 Mbp on chromosome 9 (Wang et al. 2018). These findings are indicative of a likely common genetic basis for the heterosis for kernel-related traits between PA × PB and PA × TSPT, and may be useful for future maize breeding based on marker-assisted selection. Additionally, overlapping regions were detected for the trait means and the MPH in different studies. The heterotic clusters QC2-4 and QC2-5 were co-localized with the meta-QTLs for kernel-related traits reported by Chen et al. (2017). Moreover, QC2-5 for the MPH for KT and VW overlapped with *qKD2b* for kernel density between 209 and 215 Mbp on chromosome 2 at 29 days after pollination (Zhang et al. 2016). Furthermore, the QC8 region for the MPH for KT, HKW, and WKEL overlapped with *qKD8a* for kernel density near position 164 Mbp on chromosome 8 at 50 days after pollination (Zhang et al. 2016). This region also includes the meta-QTLs for grain yield, ear-related traits, and kernel-related traits described by Chen et al. (2017). Similarly, the QC10 region, which is associated with the MPH for KL and WKEL, was previously identified as a region influencing grain yield, ear-related traits, and kernel-related traits (Chen et al. 2017) and was mapped to an overlapping and pleiotropic region affecting kernel weight and kernel density at three kernel-development stages (Zhang et al. 2016). These findings imply there may be some overlap in the genetic mechanisms underlying kernel-related traits and the heterosis for these traits. Furthermore, the heterotic loci for final kernel size may be associated with the loci responsible for kernel development.

In summary, heterotic loci at the single-locus level with a low degree of dominance are considerably important for the heterosis for kernel-related traits in the IF_2 population analyzed in this study. We also determined that the genetic mechanisms influencing kernel-related traits and the MPH for these traits are likely not completely independent. Additionally, pleiotropic and heterotic regions (bins 2.03, 6.04–6.05, and 9.01–9.02) were repeatedly observed across the heterotic patterns 'Reid × PB' and 'Reid × TSPT' in an earlier study. These findings may provide important insights into the genetic basis for the MPH for kernel-related traits. These results related to the heterosis for kernel size may help to characterize the heterosis for total grain yield and

contribute to the genetic dissection of heterosis in future studies.

Acknowledgements We thank all students for participating in the fieldwork. We thank Liwen Bianji, Edanz Editing China (www.liwenbianji.cn/ac) for editing the English text of a draft of this manuscript.

Funding This study was funded by the Applied Basic Research Programs of Science and Technology Department of Sichuan Province (2016JY0065).

Data availability All supporting data and materials are available from the corresponding authors upon request.

Compliance with ethical standards

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

Research involving human participants and/or animals This article does not contain any studies with human participants or animals performed by any of the authors.

References

Bates D, Mächler M, Bolker B, Walker S (2015) Fitting linear mixed-effects models using lme4. J Stat Softw 67:1–48

Bruce AB (1910) The Mendelian theory of heredity and the augmentation of vigor. Science 32:627–628

Chen DH, Ronald PC (1999) A rapid DNA minipreparation method suitable for AFLP and other PCR applications. Plant Mol Biol Rep 17:53–57

Chen J, Zhang L, Liu S, Li Z, Huang R, Li Y, Cheng H, Li X, Zhou B, Wu S, Chen W, Wu J, Ding J (2016a) The genetic basis of natural variation in kernel size and related traits using a four-way cross population in maize. PLoS One 11:e0153428

Chen L, Li YX, Li C, Wu X, Qin W, Li X, Jiao F, Zhang X, Zhang D, Shi Y, Song Y, Li Y, Wang T (2016b) Fine-mapping of *qGW4.05*, a major QTL for kernel weight and size in maize. BMC Plant Biol 16:81

Chen L, An Y, Li Y, Li C, Shi Y, Song Y, Zhang D, Wang T, Li Y (2017) Candidate loci for yield-related traits in maize revealed by a combination of meta QTL analysis and regional association mapping. Front Plant Sci 8:2190

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

Davenport CB (1908) Degeneration, albinism and inbreeding. Science 28:454–455

Dickinson DB, Preiss J (1969) Presence of ADP-glucose pyrophosphorylase in shrunken-2 and brittle-2 mutants of maize endosperm. Plant Physiol 44:1058–1062

Doerge RW, Churchill GA (1996) Permutation tests for multiple loci affecting a quantitative character. Genetics 142:285–294

Duvick DN (1999) Heterosis: feeding people and protecting natural resources. In: Coors JG, Pandey S (eds) Proceedings of the international symposium on the genetics and exploitation of heterosis in crops, CIMMYT, Mexico city, 17–22 Aug 1997. ASA, CSSA, and SSSA, Madison, pp 19–29

East EM (1908) Inbreeding in corn. Conn Agric Exp Stn Rep 1907:419–428

Falconer DS, Mackay TFC (1996) Introduction to quantitative genetics, 4th edn. Longman, Essex

Flint-Garcia SA, Buckler ES, Tiffin P, Ersoz E, Springer NM (2009) Heterosis is prevalent for multiple traits in diverse maize germplasm. PLoS One 4:e7433

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

Garcia AAF, Wang SC, Melchinger AE, Zeng ZB (2008) Quantitative trait loci mapping and the genetic basis of heterosis in maize and rice. Genetics 180:1707–1724

Guo T, Yang N, Tong H, Pan Q, Yang X, Tang J, Wang J, Li J, Yan J (2014) Genetic basis of grain yield heterosis in an "immortalized F_2" maize population. Theor Appl Genet 127:2149

Hallauer AR, Mirando FJB (1988) Quantitative genetics in maize breeding, 2nd edn. Iowa State University, Ames

Hua JP, Xing YZ, Xu CG, Sun XL, Yu SB, Zhang QF (2002) Genetic dissection of an elite rice hybrid revealed that heterozygotes are not always advantageous for performance. Genetics 162:1885–1895

Hua JP, Xing YZ, Wei WR, Xu CG, Sun XL, Yu SB, Zhang QF (2003) Single-locus heterotic effects and dominance by dominance interactions can adequately explain the genetic basis of heterosis in an elite rice hybrid. Proc Natl Acad Sci USA 100:2574–2579

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

Kosambi DD (1943) The estimation of map distances from recombination values. Ann Eugen 12:172–175

Lan T, He K, Chang L, Cui T, Zhao Z, Xue J, Liu J (2018) QTL mapping and genetic analysis for maize kernel size and weight in multi-environments. Euphytica 214:119

Larièpe A, Mangin B, Jasson S, Combes V, Dumas F, Jamin P, Lariagon C, Jolivot D, Madur D, Fiévet J, Gallais A, Dubreuil P, Charcosset A, Moreau L (2012) The genetic basis of heterosis: multiparental quantitative trait loci mapping reveals contrasted levels of apparent overdominance among traits of agronomical interest in maize (*Zea mays* L.). Genetics 190:795–835

Li H, Ye G, Wang J (2007) A modified algorithm for the improvement of composite interval mapping. Genetics 175:361–374

Li H, Ribaut JM, Li Z, Wang J (2008) Inclusive composite interval mapping (ICIM) for digenic epistasis of quantitative traits in biparental populations. Theor Appl Genet 116:243–260

Li Y, Wang Y, Shi Y, Song Y, Wand T, Li Y (2009) Correlation analysis and QTL mapping for traits of kernel structure and yield components in maize. Sci Agric Sin 42:408–418

Li Q, Li L, Yang X, Warburton ML, Bai G, Dai J, Li J, Yan J (2010a) Relationship, evolutionary fate and function of two maize co-orthologs of rice *GW2* associated with kernel size and weight. BMC Plant Biol 10:143

Li Q, Yang X, Bai G, Warburton ML, Mahuku G, Gore M, Dai J, Li J, Yan J (2010b) Cloning and characterization of a putative *GS3* ortholog involved in maize kernel development. Theor Appl Genet 120:753

Li X, Li Y, Chen L, Wu X, Qin W, Song Y, Zhang D, Wang T, Li Y, Shi Y (2016) Fine mapping of *qKW7*, a major QTL for kernel weight and kernel width in maize, confirmed by the combined analytic approaches of linkage and association analysis. Euphytica 210:221

Li H, Yang Q, Gao L, Zhang M, Ni Z, Zhang Y (2017) Identification of heterosis-associated stable QTLs for ear-weight-related traits in an elite maize hybrid zhengdan 958 by design III. Front Plant Sci 8:561

Liu Y, Wang L, Sun C, Zhang Z, Zheng Y, Qiu F (2014) Genetic analysis and major QTL detection for maize kernel size and weight in multi-environments. Theor Appl Genet 127:1019–1037

Liu Y, Hou X, Xiao Q, Yi Q, Bian S, Hu Y, Liu H, Zhang J, Hao X, Cheng W, Li Y, Huang Y (2016) Genetic analysis in maize foundation parents with mapping population and testcross population: Ye478 carried more favorable alleles and using QTL information could improve foundation parents. Front Plant Sci 7:1417

Liu J, Huang J, Guo H, Lan L, Wang H, Xu Y, Yang X, Li W, Tong H, Xiao Y, Pan Q, Qiao F, Raihan MS, Liu H, Zhang X, Yang N, Wang X, Deng M, Jin M, Zhao L, Luo X, Zhou Y, Li X, Zhan W, Liu N, Wang H, Chen G, Li Q, Yan J (2017) The conserved and unique genetic architecture of kernel size and weight in maize and rice. Plant Physiol 175:774–785

Lu H, Romero-Severson J, Bernardo R (2003) Genetic basis of heterosis explored by simple sequence repeat markers in a random-mated maize population. Theor Appl Genet 107:494–502

Mathias L (2012) MapDisto: fast and efficient computation of genetic linkage maps. Mol Breed 30:1231–1235

Melchinger AE, Utz HF, Piepho HP, Zeng ZB, Schön CC (2007) The role of epistasis in the manifestation of heterosis: a systems-oriented approach. Genetics 177:1815–1825

Meng L, Li H, Zhang L, Wang J (2015) QTL IciMapping: integrated software for genetic linkage map construction and quantitative trait locus mapping in biparental populations. Crop J 3:269–283

Pan D, Nelson OE (1984) A debranching enzyme deficiency in endosperms of the *Sugary-7* mutants of maize. Plant Physiol 74:324–328

Powers L (1944) An expansion of Jones's theory for the explanation of heterosis. Am Nat 78:275–280

Qin WW, Li YX, Wu X, Li X, Chen L, Shi YS, Song YCH, Zhang DF, Wang TY, Li Y (2016) Fine mapping of *qKL1.07*, a major QTL for kernel length in maize. Mol Breed 36:1–9

R Development Core Team (2010) R: a language and environment for statistical computing. http://www.r-project.org. Accessed 1 Mar 2019

Rice WR (1989) Analyzing tables of statistical tests. Evolution 43:223–225

SAC (2009) National standards of the P. R. C. Standardization Administration of the People Republic of China, Standards Press of China, Beijing, P. R. China. https://www.chinesestandard.net/PDF/English.aspx/GB1353–2009. Accessed 1 Mar 2019

Samayoa LF, Malvar RA, Butrón A (2017) QTL for maize midparent heterosis in the heterotic pattern american Dent × European flint under corn borer pressure. Front Plant Sci 8:573

Schmidt RJ, Burr FA, Burr B (1987) Transposon tagging and molecular analysis of the maize regulatory locus *opaque-2*. Science 238:960–963

Schmidt RJ, Ketudat M, Aukerman MJ, Hoschek G (1992) Opaque-2 is a transcriptional activator that recognizes a specific target site in 22 kD zein genes. Plant Cell 4:689–700

Schön CC, Dhillon BS, Utz HF, Melchinger AE (2010) High congruency of QTL positions for heterosis of grain yield in three crosses of maize. Theor Appl Genet 120:321–332

Shull GH (1908) The composition of a field of maize. Am Breed Assoc Rep 4:296–301

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

Tang J, Yan J, Ma X, Teng W, Wu W, Dai J, Dhillon BS, Melchinger AE, Li J (2010) Dissection of the genetic basis of heterosis in an elite maize hybrid by QTL mapping in an immortalized F_2 population. Thero Appl Genet 120:333–340

Wang D, Zhu J, Li Z, Paterson A (1999) Mapping QTLs with epistatic effects and QTL × environment interactions by mixed linear model approaches. Theor Appl Genet 99:1255–1264

Wang H, Zhang X, Yang H, Liu X, Li H, Yuan L, Li W, Fu Z, Tang J, Kang D (2016) Identification of heterotic loci associated with grain yield and its components using two CSSL test populations in maize. Sci Rep 6:38205

Wang Y, Zhang X, Shi X, Sun C, Jin J, Tian R, Wei X, Xie H, Guo Z, Tang J (2018) Heterotic loci identified for maize kernel traits in two chromosome segment substitution line test populations. Sci Rep 8:11101

Wei X, Lu X, Zhang Z, Xu M, Mao K, Li W, Wei F, Sun P, Tang J (2016) Genetic analysis of heterosis for maize grain yield and its components in a set of SSSL testcross populations. Euphytica 210:181

Williams W (1959) Heterosis and the genetics of complex characters. Nature 184:527–530

Yang J, Zhu J, Williams RW (2007) Mapping the genetic architecture of complex traits in experimental populations. Bioinformatics 23:1527–1736

Yang J, Hu C, Hu H, Yu R, Xia Z, Ye X, Zhu J (2008) QTLNetwork: mapping and visualizing genetic architecture of complex traits in experimental populations. Bioinformatics 24:721–723

Yang C, Zhang L, Jia A, Rong T (2016) Identification of QTL for maize grain yield and kernel-related traits. J Genet 95:239–247

Zhan J, Wang F, Xing W, Liu J, Fan Z, Tao Y (2018) Fine mapping and candidate gene prediction of a major QTL for kernel number per ear in maize. Mol Breed 38:27

Zhang Z, Liu Z, Hu Y, Li W, Fu Z, Ding D, Li H, Qiao M, Tang J (2014) QTL Analysis of kernel-related traits in maize using an immortalized F_2 population. PLoS One 9:e89645

Zhang Z, Wu X, Shi C, Wang R, Li S, Wang Z, Liu Z, Xue Y, Tang G, Tang J (2016) Genetic dissection of the maize kernel development process via conditional QTL mapping for three developing kernel-related traits in an immortalized F_2 population. Mol Genet Genomics 291:437–454

Zhang C, Zhou Z, Yong H, Zhang X, Hao Z, Zhang F, Li M, Zhang D, Li X, Wang Z, Weng J (2017) Analysis of the genetic architecture of maize ear and grain morphological traits by combined linkage and association mapping. Theor Appl Genet 130:1011–1029

Zhou Q, Dong Y, Shi Q, Zhang L, Chen H, Hu C, Li Y (2017) Verification and fine mapping of *qGW1.05*, a major QTL for grain weight in maize (*Zea mays* L.). Mol Genet Genomics 292:871

Zhu X, Shao X, Pei Y, Guo X, Li J, Song X, Zhao M (2018) Genetic diversity and genome-wide association study of major ear quantitative traits using high-density SNPs in maize. Front Plant Sci 9:966