Maize (Zea mays L.) is an important crop for food, feed, and fuel worldwide, and improving grain yield is a top priority in current maize breeding programs (Li et al. 2011b, Qin et al. 2016, Su et al. 2017). Maize grain yield is determined by several secondary components, among which the kernel size, as assessed by kernel length (KL), kernel width (KW), and kernel thickness (KT), plays a key role in determining kernel weight, and thus grain yield (Chen et al. 2016c, Gupta et al. 2006, Liu et al. 2014, Raihan et al. 2016). The kernel size also affects grain filling, as well as seedling vigor and end-use quality, which can influence the market grade and consumer preference (Chen et al. 2016a, Liu et al. 2011, Raihan et al. 2016, Revilla et al. 1999). The maize kernel-related traits are classic quantitative traits with complex genetic mechanisms that are under the control of quantitative trait loci (QTLs) with small effects and under the influence of environmental changes (Chen et al. 2016b, Qin et al. 2016, Raihan et al. 2016). Thus, understanding the genetic mechanisms of maize kernel-related traits is critical for the genetic manipulation of grain yield.

With the advent of crop genomics and development of molecular markers, QTL-based approaches have been proven to be powerful tools for elucidating the genetic basis of kernel-related traits, thus allowing improvements in high-yield breeding efficiency (Chen et al. 2016b, Li et al. 2011a, Monaco et al. 2013, Raihan et al. 2016). In rice, several genes (or QTLs) for kernel-related traits have been isolated and functionally characterized using map-based cloning (Huang et al. 2013, Li et al. 2011a, including GS2 (Zhang et al. 2013), GS3 (Fan et al. 2006), GS5 (Li et al. 2011a), GL3.1 (Qi et al. 2012), GW2 (Song et al. 2007), GW5 (Weng et al. 2008), GW7 (Wang et al. 2015), and GW8 (Wang et al. 2012). In maize, genes directly affecting kernel-related traits are classic quantitative traits with complex genetic mechanisms that are under the control of quantitative trait loci (QTLs) with small effects and under the influence of environmental changes (Chen et al. 2016b, Qin et al. 2016, Raihan et al. 2016). Thus, understanding the genetic mechanisms of maize kernel-related traits is critical for the genetic manipulation of grain yield.
kernel-related traits were rarely identified through natural genetic variation (Chen et al. 2016c), but several genes for kernel-related traits, such as gln1-3/gln1-4 (Martin et al. 2006), rgf1 (Maitz et al. 2000), dek1 (Lid et al. 2002), sh1 and sh2 (Thévenot et al. 2005), and incw2 (Carlson and Chourey 1999), were isolated using maize mutants. In addition, four orthologues of rice GS3, GS5 and GW2, namely ZmGS3, ZmGS5, and ZmGW2-CHR4 and ZmGW2-CHR5, respectively, were isolated from maize using homology-based cloning, and determined to be associated with kernel-related traits (Li et al. 2010a, 2010b, Liu et al. 2015). These genes facilitated the dissection of kernel development and its regulation (Chen et al. 2016c). Compared with mutant effects and homology-based cloning, the QTL-based mapping approach is ideal to detect favorable QTLs/genes underlying the natural variations in kernel-related traits (Chen et al. 2016a). Considerable effort has been applied to dissect the genetic variation of grain yield, and many QTLs associated with kernel-related traits in maize have been identified (Chen et al. 2016a, 2016b, 2016c, Li et al. 2013, 2016, Liu et al. 2014, Ma et al. 2007, Messmer et al. 2009, Qin et al. 2016, Raihan et al. 2016, Su et al. 2017, Yang et al. 2016, Zhang et al. 2014, 2017, Zhou et al. 2017). However, such QTLs, mainly identified by linkage analysis based on bi-parental populations, were only partially consistent across various studies using different populations, suggesting the strong influence of the genetic background. In addition, due to the large confidence intervals of the QTLs (caused by the limited recombination events) and the restricted alleles in the bi-parental genotypes, very few QTLs have been incorporated into maize high-yield breeding programs (Hao et al. 2015, Riedelsheimer et al. 2012).

The technical advances in next-generation sequencing and the development of improved statistical methods have enabled using genome-wide association studies (GWAS) based on linkage disequilibrium (LD) analyses as powerful alternatives for examining quantitative traits in plants (Xiao et al. 2017). Compared with linkage mapping based on bi-parental populations, GWAS has the potential to exploit nearly all recombination events in the evolutionary history of a specific germplasm, providing increased map resolution, and it can simultaneously evaluate the varying effects of multiple alleles (Lu et al. 2010). The key constraints for the successful use of GWAS in plants are related to population structure and rare alleles (low-frequency functional alleles), which severely limit the power of QTL detection (Gupta et al. 2005, Lu et al. 2010). However, the combination of linkage mapping and GWAS is able to effectively overcome some of the inherent limitations of each method and has been successfully applied to identify QTLs underlying drought tolerance, male inflorescence size, and grain morphology in maize (Li et al. 2016, Lu et al. 2010, Zhang et al. 2017).

In the present study, a maize recombinant inbred line (RIL) population, derived from ‘DH1M’ × ‘T877’, and a set of 253 elite maize inbred lines from modern breeding programs were evaluated to detect the QTLs underlying maize kernel-related traits based on high-density skeleton bin map, and single nucleotide polymorphism (SNP) loci associated with kernel-related traits using GWAS. Consistent QTLs for kernel-related traits were identified by combining linkage mapping and GWAS. Information on the QTLs or SNP loci involved in the kernel-related traits identified in this study will facilitate breeding strategies for grain yield improvement in maize.

Materials and Methods

Materials and phenotypic evaluation

The RIL population used here consisting of 204 lines (Li et al. 2018) produced by single-seed descent, and derived from a cross between two elite maize inbred lines, ‘DH1M’ and ‘T877’, in Chinese maize breeding programs (Xue et al. 2010, Zhao et al. 2001). These two parental lines differ significantly in kernel size and grain yield, thus providing materials to examine the genetic basis of maize kernel-related traits. The 204 RILs and the two parental lines were evaluated in Nantong and Sanya (both in China) in 2016 (Nantong only) and 2017. Each location plus year combination was considered as an environment: Nantong 2016 and 2017 were designated as environments E1 and E2, respectively, while Sanya 2017 was designated as E3.

A set of 253 elite maize inbred lines with abundant phenotypic variation, including traditional landraces and improved maize inbred lines selected from a wide range of geographical locations in China (Supplemental Table 1), were collected to construct an association panel for GWAS (Zhou et al. 2016a). This association panel was evaluated in Nantong in 2016 and 2017, which were designated as environment E1’ (Nantong 2016) and E2’ (Nantong 2017), respectively.

Two populations were arranged following a randomized complete block design with three replicates in all environments. Each genotype was grown in a single row (500-cm long; 60-cm between rows), each with 20 plants, and standard agronomic practices were performed in each environment. Five representative well opened pollinated ears per line and per replicate were harvested for phenotypic measurements after maturity. Four kernel-related traits, namely kernel length (KL, mm), kernel width (KW, mm), kernel thickness (KT, mm), and 100-kernel weight (HKW, g), were evaluated in all environments. KL, KW, and KT were estimated from the averages of 10 randomly selected kernels from the center of each ear using an electronic digital caliper. Whereas HKW was measured as the average weight of three samples of 100 mixed seeds from five ears.

Phenotypic data analyses

Statistical analyses of all phenotypic data of the tested traits across different environments were performed using SAS 9.13 software (SAS Institute, Cary, NC, USA). The broad-sense heritability (HF) of each trait was estimated
according to the formula: $H^2 (%) = \frac{\sigma^2_g}{\sigma^2_g + \sigma^2_ge + \sigma^2_e/n} \times 100\%$, where $\sigma^2_g$ is the genotypic variance, $\sigma^2_{ge}$ is the variance for interactions of genotype with environment, $\sigma^2_e$ is the error variance, n is the number of environments, and r is the number of replications (Hallauer et al. 2010), which were estimated using the PROC VARIOPTION procedure in SAS. To minimize the effects of environmental factors, the best linear unbiased predictions (BLUPs) for each trait in each maize inbred line across environments were evaluated using the PROC MIXED procedure in SAS. Pearson correlation coefficients between the tested traits were calculated using the PROC CORR procedure in SAS, based on the BLUPs of the traits across environments.

### Results

**Phenotypic variations and correlations**

Phenotype variations of kernel-related traits in the two mapping populations are presented in Tables 1 and 2. All four tested traits varied widely in both the RIL and the association mapping populations. For example, the BLUPs for KW ranged from 6.69 to 9.78 mm in the RIL population, and from 6.42 to 10.76 mm in the association mapping population; the BLUPs for HKW ranged from 14.88 to 31.87 g in the RIL population, and from 8.68 to 31.60 g in the association mapping population. An analysis of variance for all tested kernel-related traits suggested that the genotypic and genotype-by-environmental effects were highly significant at the 0.001 probability level in both populations. The estimated $H^2$ of the kernel-related traits ranged from 90.52 for KT to 96.47 for KW in the RIL population, and from 79.34 for KL to 93.44 for KW in the association mapping population.

The four traits in the two populations were significantly correlated with each other, except for KL and KT in the RIL population (Table 3). Significant positive correlations were observed between HKW and the other three traits, with correlation coefficient ($r$) values of 0.400, 0.631, and 0.433 for KL, KW, and KT, respectively, in the RIL population, and of 0.516, 0.744, and 0.589 for KL, KW, and KT, respectively, in the association mapping population.

### QTL mapping of kernel-related traits in the RIL population

Twenty-seven QTLs were detected for the four kernel-related traits across the three different environments and the BLUPs across all of the environments, which were distributed on eight maize chromosomes, excluding chromosome 7 and 9 (Table 4). In total, three QTLs underlying KL were located on chromosomes 3, 5, and 8. The 15 QTLs for KW were distributed on seven chromosomes, but none were found on chromosomes 7, 8, and 9. The 9 QTLs for HKW were located on chromosome 1, 2, 3, 4, and 5. However, no QTLs were detected for KT. The percentage of phenotypic variance explained by the individual QTLs ranged from 3.34% (KW, qKW2-2) to 11.84% (KW, qKW10-1) for the different traits (Table 4). Of these QTLs, 14 (~50.0%) showed positive additive effects, which indicated that the favorable alleles from ‘DH1M’ could increase the values of the test traits.
tent QTLs were detected for KL or KT. A QTL for KW (qKW1-3 located in the marker interval of PZE-101066217_SYN29479 on chromosome 1) and a QTL for HKW (qHKW1-1 flanked by SYN33163 and SYN367 on chromosome 1) were stably identified in all different environments and across environments. The positive additive effects of these consistent QTLs for KW and HKW were contributed by the favorable alleles from the ‘DH1M’ parent, which had large KW and HKW.

Among these detected QTLs, only four (designated as ‘consistent’ QTLs) were stably detected in two or more environments as well as across environments (Table 4). Of these consistent QTLs, two were stably identified for KW (on chromosomes 1 and 4) and two were stably identified for HKW (on chromosomes 1 and 2). However, no consis-

Table 1. Descriptive statistics, analysis of variance, and broad-sense heritability for maize kernel-related traits in the recombinant inbred line (RIL) population and parental lines across three environments

| Traits | Env. | RIL population Mean ± SD | Range | Parental lines (mean ± SD) T877 | G | G × E | H² (%) |
|--------|------|--------------------------|-------|--------------------------------|---|------|-------|
| KL (mm) | E1   | 9.08 ± 0.68 | 7.08–11.45 | 8.63 ± 0.09 | 9.40 ± 0.34 | ** | ** | 92.74 |
| E2     | 9.09 ± 0.56 | 7.12–11.01 | 9.29 ± 0.22 | 9.78 ± 0.21 | | | |
| E3     | 9.33 ± 0.68 | 7.09–11.60 | 8.88 ± 0.17 | 9.55 ± 0.24 | | | |
| BLUPS  | 9.16 ± 0.55 | 7.23–11.19 | 8.94 ± 0.33 | 9.58 ± 0.19 | | | |
| KW (mm) | E1   | 8.18 ± 0.57 | 6.54–10.04 | 8.34 ± 0.08 | 8.91 ± 0.15 | ** | ** | 96.47 |
| E2     | 8.11 ± 0.50 | 6.63–9.69 | 8.41 ± 0.10 | 8.80 ± 0.15 | | | |
| E3     | 8.32 ± 0.55 | 6.62–9.81 | 8.43 ± 0.15 | 8.96 ± 0.07 | | | |
| BLUPS  | 8.21 ± 0.49 | 6.69–9.78 | 8.40 ± 0.05 | 8.89 ± 0.08 | | | |
| KT (mm) | E1   | 5.21 ± 0.56 | 3.76–6.72 | 5.12 ± 0.13 | 4.48 ± 0.20 | ** | ** | 90.52 |
| E2     | 5.09 ± 0.48 | 4.00–6.85 | 4.99 ± 0.19 | 4.56 ± 0.27 | | | |
| E3     | 5.09 ± 0.51 | 3.76–6.69 | 4.94 ± 0.25 | 4.48 ± 0.20 | | | |
| BLUPS  | 5.13 ± 0.45 | 3.99–6.64 | 5.02 ± 0.09 | 4.50 ± 0.05 | | | |
| HKW (g) | E1   | 22.61 ± 3.58 | 13.27–34.50 | 18.23 ± 0.65 | 21.12 ± 0.89 | ** | ** | 95.04 |
| E2     | 22.60 ± 3.30 | 13.73–32.47 | 19.91 ± 0.18 | 22.82 ± 0.90 | | | |
| E3     | 23.52 ± 3.53 | 13.27–32.67 | 18.20 ± 0.89 | 21.37 ± 0.42 | | | |
| BLUPS  | 22.92 ± 3.11 | 14.88–31.87 | 18.78 ± 0.98 | 21.77 ± 0.92 | | | |

** Significant at P < 0.001.

Table 2. Descriptive statistics, analysis of variance, and broad-sense heritability for maize kernel-related traits in 253 elite maize inbred lines across two environments

| Traits | Env. | Mean ± SD | Range | G | G × E | H² (%) |
|--------|------|-----------|-------|---|------|-------|
| KL (mm) | E1    | 8.82 ± 1.01 | 6.46–12.96 | ** | 79.34 |
| E2    | 9.16 ± 0.81 | 6.22–11.62 | | | |
| BLUPS  | 9.00 ± 0.73 | 7.14–11.85 | | | |
| KW (mm) | E1    | 8.08 ± 0.81 | 5.98–11.96 | ** | 93.44 |
| E2    | 8.25 ± 0.70 | 5.86–10.50 | | | |
| BLUPS  | 8.18 ± 0.64 | 6.42–10.76 | | | |
| KT (mm) | E1    | 4.64 ± 0.54 | 3.13–7.23 | ** | 84.10 |
| E2    | 4.76 ± 0.49 | 3.33–6.65 | | | |
| BLUPS  | 4.70 ± 0.38 | 3.84–6.09 | | | |
| HKW (g) | E1    | 19.09 ± 5.91 | 5.48–34.15 | ** | 92.72 |
| E2    | 21.09 ± 4.08 | 8.42–31.54 | | | |
| BLUPS  | 20.14 ± 4.28 | 8.68–31.60 | | | |

** Significant at P < 0.001.

Table 3. Correlations among maize kernel-related traits in the recombinant inbred lines (RILs) and the association panel

| Traits | KL | KW | KT | HKW |
|--------|----|----|----|-----|
| KL    | 0.448** | –0.057 | 0.400** |
| KW    | 0.440** | 0.201** | 0.631** |
| KT    | 0.194*  | 0.515** | 0.433** |
| HKW   | 0.516** | 0.744** | 0.589** |

Correlation coefficients for the RIL population are above the diagonal, while those for the association panel are below the diagonal. ** Significant at P < 0.001.

GWAS of kernel-related traits

Overall, 2,824 high-quality SNPs with missing data ≤ 20% and MAF ≥ 5% in the association mapping population were used for the GWAS performed here. Based on the MLM incorporating Q + K, 461 marker-trait associations among these 27 QTLs, two QTLs of qHKW1-1 for HKW and qKW1-2 for KW were co-localized in the same marker interval of SYN33163 and SYN367 on chromosome 1.
Table 4. QTLs detected for maize kernel-related traits in the recombinant inbred line (RIL) population in three environments and the best linear unbiased predictions (BLUPs) across environments

| Traits | QTL | Chr | Position (cM) | Marker interval | E1 LOD | E1 PVE (%) | Add | E2 LOD | E2 PVE (%) | Add | E3 LOD | E3 PVE (%) | Add | BLUPs LOD | BLUPs PVE (%) | Add |
|--------|-----|-----|---------------|----------------|-------|------------|-----|-------|------------|-----|-------|------------|-----|-----------|----------------|-----|
| KL     | qKL3-1 | 3   | 125           | PZE-103049569_PZE-103051543 | 3.41  | 5.43       | -0.13 | 5.77  | 6.20       | -0.13 |
|        | qKL5-1 | 5   | 183           | SYN35495_PZE-105110743 | 6.06  | 10.23      | -0.18 | 4.45  | 4.61       | 0.11  |
|        | qKL8-1 | 8   | 157           | PZE-108069615_PZE-108070036 | 3.74  | 5.96       | 0.14  | 5.81  | 6.34       | 0.13  |
| KW     | qKW1-1 | 1   | 27            | SYN7706_SYN6742 | 6.35  | 6.59       | -0.16 | 8.86  | 10.33      | 0.17  |
|        | qKW2-1 | 1   | 61            | SYN33163_SYN367 | 7.55  | 8.30       | 0.18  | 4.45  | 4.61       | 0.11  |
|        | qKW3-1 | 3   | 121           | PZE-101066217_SYN29479 | 4.82  | 4.86       | 0.14  | 5.65  | 8.38       | 0.16  |
|        | qKW2-1 | 2   | 87            | SYN451_PZE-102039760 | 3.40  | 3.34       | 0.11  | 6.91  | 10.31      | 0.18  |
|        | qKW2-2 | 2   | 118           | SYNGENTA3962_PZE-102065594 | 3.40  | 3.34       | 0.11  | 3.85  | 3.96       | -0.10 |
|        | qKW2-3 | 2   | 145           | PZE-102108955_PZE-102109699 | 3.58  | 5.12       | 0.11  | 4.44  | 5.35       | 0.12  |
|        | qKW2-4 | 2   | 167           | PZE-102126983_SYN33456 | 5.12  | 7.79       | 0.16  | 4.35  | 3.98       | 0.11  |
|        | qKW2-5 | 5   | 129           | PZE-105062861_SYN1318 | 5.96  | 6.12       | -0.15 | 4.91  | 7.17       | -0.15 |
|        | qKW2-6 | 5   | 184           | PZE-105111506_PZE-10513106 | 6.38  | 9.38       | -0.15 | 3.85  | 3.96       | -0.10 |
|        | qKW2-7 | 5   | 210           | PZE-105130823_PZE-105132493 | 4.28  | 6.21       | -0.84 | 4.57  | 6.39       | -0.84 |
| HKW    | qHKW1-1 | 1   | 60            | SYN33163_SYN367 | 5.67  | 8.97       | 1.14  | 5.38  | 9.46       | 1.10  |
|        | qHKW2-1 | 1   | 296           | SYNI5632_PZE-10117851 | 3.90  | 5.21       | 0.77  | 6.22  | 10.08      | 1.03  |
|        | qHKW3-1 | 1   | 98            | SYNI624_SYN635 | 5.68  | 8.77       | 1.13  | 4.33  | 7.56       | 0.99  |
|        | qHKW2-2 | 2   | 124           | PZE-103000497_PZE-103010477 | 5.18  | 7.03       | 0.89  | 3.77  | 5.03       | 0.75  |
|        | qHKW2-3 | 2   | 145           | PZE-104087825_PZE-104088242 | 5.96  | 6.12       | -0.15 | 3.46  | 5.60       | -0.87 |
|        | qHKW2-4 | 4   | 210           | PZE-105062861_SYN1318 | 5.96  | 6.12       | -0.15 | 3.46  | 5.60       | -0.87 |
|        | qHKW5-1 | 5   | 129           | PZE-105062861_SYN1318 | 5.96  | 6.12       | -0.15 | 3.46  | 5.60       | -0.87 |
|        | qHKW5-2 | 5   | 210           | PZE-105130823_PZE-105132493 | 4.28  | 6.21       | -0.84 | 4.57  | 6.39       | -0.84 |
|        | qHKW5-3 | 5   | 210           | PZE-105130823_PZE-105132493 | 4.00  | 5.73       | -0.91 | 3.71  | 5.72       | -0.77 |

KL, kernel length; KW, kernel width; HKW, 100-kernel weight.

QTLs identified are named with trait abbreviations followed by the chromosome number.

LOD, log₁₀ of odds ratio.

PVE, percentage of phenotypic variance explained by a single QTL.

Add, additive effect.
involving 231 SNPs, representing all chromosomes, were identified as being associated with the four kernel-related traits in different environments and the BLUPs across environments at the threshold level of $P \leq 0.01$ ($-\log P \geq 2.00$) (Supplemental Figs. 1, 2). After correction for multiple testing using the FDR method ($FDR \leq 0.05$), 40 marker-trait associations representing 29 SNPs remained significant, these SNPs were distributed among 8 chromosomes, excluding chromosome 5 and 9 (Supplemental Table 2). Of these 29 SNPs, nine were significantly associated with KW, KT, and HKW, in at least one environment as well as the BLUPs across environments (Table 5). Two SNPs of PZE-101066560 on chromosome 1 were stably identified for KW and HKW, respectively, in both environments and across environments.

After correction for multiple testing using the FDR method ($FDR \leq 0.05$), two SNPs showed pleiotropy with different traits in this association panel (Supplemental Table 2), PZE-101066560 on chromosome 1 and PZE-103033919 on chromosome 3 were co-associated with KW and HKW.

### Discussion

Yield improvement is a top priority for maize breeding (Chen et al. 2016b). Kernel size and kernel weight are important components of maize yield, and the latter is largely determined by kernel size, which has been strongly selected during maize domestication and improvement (Gupta et al. 2006, Liu et al. 2017, Raihan et al. 2016). In the present study, wide variations in kernel-related traits were observed in both the RIL population and the association panel, and significant positive correlations were also observed between HKW and the other three kernel size traits in both populations. This indicated that kernel size plays an important role in determining HKW, suggesting that these traits might be simultaneously improved in breeding programs (Zhang et al. 2017). Moreover, the correlation coefficients for KW were usually greater than those for KL and KT in both evaluated populations, as found by Liu et al. (2017), suggesting that KW might play a more important role than other traits in determining kernel weight in maize. The estimates of $H^2$ were high for all kernel-related traits in both evaluated populations, suggesting that genetic factors play an important role in the formation of these traits, which was consistent with the results of previous studies (Liu et al. 2014, Raihan et al. 2016). In the present study, wide variations in kernel-related traits were observed in both the RIL population and the association panel, and significant positive correlations were also observed between HKW and the other three kernel size traits in both populations. This indicated that kernel size plays an important role in determining HKW, suggesting that these traits might be simultaneously improved in breeding programs (Zhang et al. 2017). Moreover, the correlation coefficients for KW were usually greater than those for KL and KT in both evaluated populations, as found by Liu et al. (2017), suggesting that KW might play a more important role than other traits in determining kernel weight in maize. The estimates of $H^2$ were high for all kernel-related traits in both evaluated populations, suggesting that genetic factors play an important role in the formation of these traits, which was consistent with the results of previous studies (Liu et al. 2014, Raihan et al. 2016).
et al. 2016). In addition, obvious bi-directional transgressive segregation was detected for all traits in the RIL population, indicating their polygenic control. It is important to map QTLs for kernel-related traits to increase our understanding of the genetic and molecular bases of maize grain yield, which will facilitate marker-assisted selection (MAS) to select for the genetic determinant(s) of maize yield.

In the present study, linkage mapping and GWAS were used to dissect the candidate loci associated with the kernel-related traits of maize. Twenty-seven QTLs and 29 associated SNPs were identified for the four kernel-related traits examined here through linkage mapping and GWAS, respectively. Most of the identified QTLs or loci with small effects confirmed that the kernel-related traits in modern maize inbred lines are controlled by multiple genes with low effects, in agreement with the results of a previous study (Zhang et al. 2017). Only four of these QTLs for kernel-related traits were stably detected in at least two environments or across environments in the RIL population, and only two loci could be stably detected in both environments and across environments in the association panel, indicating that many of the QTLs/SNPs detected were environmentally specific. Thus, most QTLs for kernel-related traits of maize might be affected by environmental factors, having QTL-by-environment interactions, and controlled by multiple genes with minor effects (Hao et al. 2017, Raihan et al. 2016, Zhou et al. 2016b). However, only stable and highly heritable QTLs will be useful for the MAS aiming to increase maize yield in a wide range of environments in breeding programs (Zhou et al. 2016b). The stable QTLs identified in the present study for four kernel-related traits that have relatively high heritability levels, such as qKW1-3 and qHKW1-1, should be considered priority candidates for MAS in modern maize breeding programs. Some of the four stable QTLs identified here were located in the same regions as those identified for yield-related traits in previous studies, such as qKW1-3 for KW in bin 1.02 (Liu et al. 2014), qKW4-1 for KW in bin 4.02 (Austin and Lee 1996), and qHKW1-1 for HKW in bin 1.02 (Liu et al. 2014). The stable QTL of qHKW2-1 is a novel QTL. These results indicated that some causal gene/genes might be located in these regions, and these stable QTLs might be further used for fine mapping, gene cloning, and validation of the potential candidate genes, which may be highly valuable in maize breeding (Zhang et al. 2017, Zhou et al. 2016b).

The QTLs for domestication-related traits form clusters that are consistent with the regions harboring favorable genes (Cai and Morishima 2002, Liu et al. 2014, Zhang et al. 2017). In the present study, two QTLs from RILs and two SNPs from the association panel were associated with various traits, which shared significant correlations. Some of these QTL/SNPs were located in or near regions where QTLs for yield-related traits have been mapped in previous studies (Agrama and Moussa 1996, Liu et al. 2014). For example, a QTL cluster in the interval of SYN33163_SYN367 on chromosome 1 was responsible for two significant QTLs, qKW1-2 and qHKW1-1, in the RILs, the SNP PZE-101066560 on chromosome 1 was co-associated with KW and HKW in the association panel. The clustering or co-association of QTLs/SNPs for different kernel-related traits in the two evaluated populations could be explained by the pleiotropy of the same gene(s) or by the joint effects of closely linked genes (in local LD) in the identified regions associated with target traits, which may benefit from the association of adaptive phenotypes during domestication (Bergelson and Roux 2010, Liu et al. 2014, Marathi et al. 2012, Zhang et al. 2017). In modern breeding schemes for maize grain yield improvement, MAS of these clustered/co-associated loci might simultaneously improve multiple targeted traits.

Linkage analysis and GWAS are two complementary approaches commonly used to dissect the genetic architecture of traits of interest (Lu et al. 2010), and consistency between the QTLs from the linkage mapping and the associated loci from the GWAS could provide cross-validation (Zhang et al. 2017). In the present study, a loci of PZE-101066560 on chromosome 1 was identified as being associated with KW and HKW by GWAS, and this locus was found co-localized within the QTL interval of qKW1-3 (flanked by PZE-101066217 and SYN29479), associated with KW by linkage mapping. In this region, the QTLs for kernel size and kernel weight were identified in previous studies (Li et al. 2013, Liu et al. 2014). Twenty-three protein-encoding genes were located in the genomic region of qKW1-3, of which five were located in the LD block of PZE-101066560. According to the B73 reference genome sequence Version 5b.60 and the gene annotation data available at MaizeGDB, two annotated genes, GRMZM5G824629 (encoding a ubiquitin-conjugating enzyme) and GRMZM2G011483 (encoding a cyclin-related protein), were the most likely candidate genes for kernel width and kernel weight. In rice, GW2 and GW3 are involved in the ubiquitin-proteasome pathway that regulates grain size and weight (Song et al. 2007, Weng et al. 2008). The ubiquitin pathway may also play critical roles in grain development in other plant species (Li et al. 2008, Song et al. 2007). Kernel size might be determined by the stretching ability of the kernel, both longitudinally and latitudinally, during cell division, which affects the final endosperm and embryo sizes (Kesavan et al. 2013, Li et al. 2016). In rice, the gene/QTL GL3.1 that controls grain size and yield might also be involved in cell cycle regulation, suggesting a new mechanism for the regulation of grain size and yield that is driven by cell cycle progression (Qi et al. 2012). The candidate gene GRMZM2G011483 revealed in the present study might be involved in the same pathway for grain size control. These results provide basis for further research into the genetic mechanisms of maize kernel-related traits and for a MAS to increase kernel size in maize yield improvement programs. The fine mapping of these QTLs and further studies on the molecular functions of these potential candidate genes will provide more insights into the underlying genetic
and molecular mechanisms of maize grain development, which could be used for maize grain yield improvement.

Acknowledgments

This work was funded by the National Key Technology Research and Development Program of MOST (2016YFD0100303), the China Postdoctoral Science Foundation (2016MS51935), the Jiangsu Planned Projects for Postdoctoral Research Funds (1501116B), the Six Major Talent Project of Jiangsu Province, China (2016-NY-143), the Scientific and Technological Project of Jiangsu Province, China (BE2018325), the Jiangsu Agriculture Science and Technology Innovation Fund (CX17(2013), and the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD).

Literature Cited

Agrama, H.A.S. and M.E. Moussa (1996) Mapping QTLs in breeding for drought tolerance in maize (Zea mays L.). Euphytica 91: 89–97.

Austin, D.F. and M. Lee (1996) Comparative mapping in F_{2:3} and F_{2:7} generations of quantitative trait loci for grain yield and yield components in maize. Theor. Appl. Genet. 92: 817–826.

Bergelson, J. and F. Roux (2010) Towards identifying genes underlying ecologically relevant traits in Arabidopsis thaliana. Nat. Rev. Genet. 11: 867–879.

Bradbury, P.J., Z. Zhang, D.E. Kroon, T.M. Casstevens, Y. Ramdoss and E.S. Buckler (2007) TASSEL: software for association mapping of complex traits in diverse samples. Bioinformatics 23: 2633–2635.

Cai, W. and H. Morishima (2002) QTL clusters reflect character associations in wild and cultivated rice. Theor. Appl. Genet. 104: 1217–1228.

Carlson, S.J. and P.S. Chourey (1999) A re-evaluation of the relative roles of two invertases, INCW2 and IRV1, in developing maize kernels and other tissues. Plant Physiol. 121: 1025–1035.

Chen, J., L. Zhang, S. Liu, Z. Li, R. Huang, Y.Li, H. Cheng, X. Li, B. Zhou, S. Wu et al. (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., C. Li, Y. Li, Y. Song, D. Zhang, T. Wang, Y. Li and Y. Shi (2016b) Quantitative trait loci mapping of yield and related traits using a high-density genetic map of maize. Mol. Breeding. 36: 134.

Chen, L., Y.-x. Li, C. Li, X. Wu, W. Qin, X. Li, F. Jiao, X. Zhang, D. Zhang, Y. Shi et al. (2016c) Fine-mapping of qGW4_05, a major QTL for kernel weight and size in maize. BMC Plant Biol. 16: 81.

Deng, M., D. Li, J. Luo, Y. Xiao, H. Liu, Q. Pan, X. Zhang, M. Jin, M. Zhao and J. Yan (2017) The genetic architecture of amino acids dissection by association and linkage analysis in maize. Plant Biotechnol. J. 15: 1250–1263.

Fan, C., Y. Xing, H. Mao, T. Lu, B. Han, C. Xu, X. Li and Q. Zhang (2006) G33, a major QTL for grain length and weight and minor QTL for grain width and thickness in rice, encodes a putative transmembrane protein. Theor. Appl. Genet. 112: 1164–1171.

Ganal, M.W., G. Durstewitz, A. Polley, A. Bérard, E.S. Buckler, A. Charcosset, J.D. Clarke, E.M. Graner, M. Hansen J. Joets et al. (2011) A large maize (Zea mays L.) SNP genotyping array: development and germplasm genotyping, and genetic mapping to compare with the B73 reference genome. PLoS ONE 6: e28334.

Gao, X., L.C. Becker, D.M. Becker, J.D. Starmer and M.A. Province (2010) Avoiding the high Bonferroni penalty in genome-wide association studies. Genet. Epidemiol. 34: 100–105.

Gupta, P.K., S. Rustgi and P.L. Kulwal (2005) Linkage disequilibrium and association studies in higher plants: present status and future prospects. Plant Mol. Biol. 57: 461–485.

Gupta, P.K., S. Rustgi and N. Kumar (2006) Genetic and molecular basis of grain size and grain number and its relevance to grain productivity in higher plants. Genome 49: 565–571.

Hallauer, A.R., M.J. Carena and J.B.M. Filho (2010) Quantitative genetics in maize breeding, 6th edn. Springer, Iowa.

Hao, D., Y. Cheng, G. Chen, H. Lu, M. Shi, Z. Zhang, X. Huang, Y. Mao and L. Xue (2015) Identification of significant single nucleotide polymorphisms for resistance to maize rough dwarf disease in elite maize (Zea mays L.) inbred lines. Euphytica 203: 109–120.

Hao, D., L. Xue, J. Yuan, Z. Zhang, H. Lu, Y. Mao, M. Shi, X. Huang, G. Zhou and G. Chen (2017) Genetic dissection of starch paste viscosity characteristics in waxy maize revealed by high-density SNPs in a recombinant inbred line population. Mol. Breeding. 37: 50.

Hardy, O. and X. Vekemans (2002) SPAGeDi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. Mol. Ecol. Notes 2: 618–620.

Huang, R., L. Jiang, J. Zheng, T. Wang, H. Wang, Y. Huang and Z. Hong (2013) Genetic bases of rice grain shape: so many genes, so little known. Trends Plant Sci. 18: 218–226.

Kesavan, M., J.T. Song and H.S. Seo (2013) Seed size: a priority trait in cereal crops. Physiol. Plant. 147: 113–120.

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

Li, C., Y. Li, B. Sun, B. Peng, C. Liu, Z. Liu, Z. Yang, Q. Li, W. Tan, Y. Zhang et al. (2013) Quantitative trait loci mapping for yield components and kernel-related traits in multiple connected RIL populations in maize. Euphytica 193: 303–316.

Li, P., Y. Zhang, S. Yin, P. Zhu, T. Pan, Y. Xu, J. Wang, D. Hao, H. Fang, C. Xu et al. (2018) QTL-by-environment interaction in the response of maize root and shoot traits to different water regimes. Front Plant Sci. 9: 229.

Li, Q., L. Li, X. Yang, M.L. Warburton, G. Bai, J. Dai, J. Li and J. Yan (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., X. Yang, G. Bai, M.L. Warburton, G. Mahuku, M. Gore, J. Dai, J. Li and J. Yan (2010b) Cloning and characterization of a putative GS3 ortholog involved in maize kernel development. Theor. Appl. Genet. 120: 753–763.

Li, X., Y.X. Li, L. Chen, X. Wu, W. Qin, Y. Song, D. Zhang, T. Wang, Y. Li and Y. Shi (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: 221–232.

Li, Y., L. Zheng, F. Corke, C. Smith and M.W. Bevan (2008) Control of final seed and organ size by the DA1 gene family in Arabidopsis thaliana. Genes Dev. 22: 1331–1336.

Li, Y., C. Fan, Y. Xing, Y. Jiang, L. Luo, L. Sun, D. Shao, C. Xu, X. Li, J. Xiao et al. (2011a) Natural variation in G35 plays an important role in regulating grain size and yield in rice. Nat. Genet. 43: 1266–1269.

Li, Y., X. Ma, T. Wang, Y. Li, C. Liu, Z. Liu, B. Sun, Y. Shi, Y. Song, M. Carlone et al. (2011b) Increasing maize productivity in China by planting hybrids with germplasm that responds favorably to higher planting densities. Crop Sci. 51: 2391–2400.

Lid, S.E., D. Gruis, R. Jung, J.A. Lorentzen, E. Ananiev, M. Chamberlin, X. Niu, R. Meeley, S. Nichols and O.A. Olsen (2002) The defective kernel 1 (dek1) gene required for aleurone cell development in the endosperm of maize grains encodes a membrane protein of the...
Combined linkage and association mapping of kernel-related traits in maize

Calpain gene superfamily. Proc. Natl. Acad. Sci. USA 99: 5460–5465.

Liu, J., M. Deng, H. Guo, S. Raihan, J. Luo, Y. Xu, X. Dong and J. Yan (2015) Maize orthologs of rice GS5 and their trans-regulator are associated with kernel development. J. Integr. Plant Biol. 57: 943–953.

Liu, J., J. Huang, H. Guo, L. Lan, H. Wang, Y. Xu, X. Yang, W. Li, H. Tong, Y. Xiao et al. (2017) The conserved and unique genetic architecture of kernel size and weight in maize and rice. Plant Physiol. 175: 774–785.

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

Liu, Z., H. Ji, Z. Cui, X. Wu, L. Duan, X. Feng and J. Tang (2011) QTL detected for grain-filling rate in maize using a RIL population. Mol. Breed. 27: 25–36.

Lu, Y., S. Zhang, T. Shah, C. Xie, Z. Hao, X. Li, M. Farkhari, J.-M. Ribaut, M. Cao, T. Rong et al. (2010) Joint linkage–linkage disequilibrium mapping is a powerful approach to detecting quantitative trait loci underlying drought tolerance in maize. Proc. Natl. Acad. Sci. USA 107: 19585–19590.

Ma, X.Q., J.H. Tang, W.T. Teng, J.B. Yan, Y.J. Meng and J.S. Li (2007) Epistatic interaction is an important genetic basis of grain yield and its components in maize. Mol. Breed. 20: 41–51.

Maitz, M., G. Santandrea, Z.Y. Zhang, S. Lal, L.C. Hannah, F. Salamini et al. (2000) Inference of population structure using multilocus genotype data. Genetics 155: 945–959.

Raihan, M.S., J. Liu, J. Huang, H. Guo, S. Pan and J. Yan (2016) Multi-environment QTL analysis of grain morphology traits and fine mapping of a kernel-width QTL in Zheng58 × SK maize population. Theor. Appl. Genet. 129: 1465–1477.

Revilla, P., A. Batrón, R. Malvar and R. Ordás (1999) Relationship among kernel weight, early vigor, and growth in maize. Crop Sci. 39: 654–658.

Riedelsheimer, C., J. Lisek, A. Czedik-Eysenberg, R. Sulpice, A. Flis, C. Grieder, T. Altman, M. Stitt, L. Willmitzer and A.E. Melchinger (2012) Genome-wide association mapping of leaf metabolic profiles for dissecting complex traits in maize. Proc. Natl. Acad. Sci. USA 109: 8872–8877.

Song, X.J., W. Huang, M. Shi, M.Z. Zhu and H.X. Lin (2007) A QTL for rice grain width and weight encodes a previously unknown RING-type E3 ubiquitin ligase. Nat. Genet. 39: 623–630.

Storey, J.D., and R. Tibshirani (2003) Statistical significance for genomewide studies. Proc. Natl. Acad. Sci. USA 100: 9440–9445.

Su, C., W. Wang, S. Gong, J. Zuo, S. Li and S. Xu (2017) High density linkage map construction and mapping of yield trait QTLs in maize (Zea mays) using the genotyping-by-sequencing (GBS) technology. Front. Plant Sci. 8: 706.

Thévenot, C., E. Simond-Côte, A. Reyss, D. Manicacci, J. Trouverie, M. Le Guilloux, V. Ginhoux, F. Sidicina and J.L. Prioul (2005) QTLs for enzyme activities and soluble carbohydrates involved in starch accumulation during grain filling in maize. J. Exp. Bot. 56: 945–958.

Wang, S., K. Wu, Q. Yuan, X. Liu, Z. Liu, X. Lin, R. Zeng, H. Zhu, G. Dong, Q. Qian et al. (2012) Control of grain size, shape and quality by OsSPL16 in rice. Nat. Genet. 44: 950–954.

Wang, S., L. Li, Q. Liu, K. Wu, J. Zhang, S. Wang, Y. Wang, X. Chen, Y. Zhang, C. Gao et al. (2015) The OsSPL16-GW7 regulatory module determines grain shape and simultaneously improves rice yield and grain quality. Nat. Genet. 47: 949–954.

Weng, J., S. Gu, X. Wan, H. Gao, T. Guo, N. Su, C. Lei, X. Zhang, Z. Cheng, X. Guo et al. (2008) Isolation and initial characterization of GW5, a major QTL associated with rice grain width and weight. Cell Res. 18: 1199–1209.

Xiao, Y., H. Liu, L. Wu, M. Warburton and J. Yan (2017) Genome-wide association studies in maize: praise and stargaze. Mol. Plant 10: 359–374.

Xue, L., H.H. Liu, C.J. Peng, C.X. Sun, X.H. Chen and L.I. Bo (2010) Evaluation of new germplasm T877 resistance to maize rough dwarf disease. Plant Genet. Resour. 11: 806–810.

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

Yu, J., G. Pressoir, W.H. Briggs, I. Vroh Bi, M. Yamaseki, J.F. Doebley, M.D. McMullen, B.S. Gaut, D.M. Nielsen, J.B. Holland et al. (2006) A unified mixed-model method for association mapping that accounts for multiple levels of relatedness. Nat. Genet. 38: 203–208.

Zhang, C., Z. Zhou, H. Yong, X. Zhang, Z. Hao, F. Zhang, M. Li, D. Zhang, X. Li and Z. Wang (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.

Zhang, W., P. Sun, Q. He, F. Shu, J. Wang and H. Deng (2013) Fine mapping of GS2, a dominant gene for big grain rice. Crop J. 1: 160–165.

Zhang, Z., Z. Liu, Y. Hu, W. Li, Z. Fu, D. Ding, H. Li, M. Qiao and J. Tang (2014) QTL analysis of kernel-related traits in maize using an immortalized F2 population. PLoS ONE 9: e89645.

Zhao, B., X.A. Hu, L.Z. Jia, F.Z. Zhao and X.J. Chu (2001) Performance of new maize variety of Denghai 1 with the characteristics of high and stable yield and high resistance to disease. J. Henan Agri. Sci. 3: 8.

Zhou, G., D. Hao, G. Chen, H. Lu, M. Shi, Y. Mao, Z. Zhang, X. Huang and L. Xue (2016a) Genome-wide association study of the husk number and weight in maize (Zea mays L.). Euphytica 210: 195–205.

Zhou, Q., Y. Dong, Q. Shi, Z. Long, H. Chen, C. Hu and Y. Li (2017) Verification and fine mapping of qGW1.05, a major QTL for grain weight in maize (Zea mays L.). Mol. Genet. Genomics 292: 871–881.

Zhou, Z., C. Zhang, Y. Zhou, Z. Hao, Z. Wang, X. Zeng, H. Di, M. Li, D. Zhang, H. Yong et al. (2016b) Genetic dissection of maize plant architecture with an ultra-high density bin map based on recombinant inbred lines. BMC Genomics 17: 178.