

QTL analysis and fine mapping of a major QTL conferring kernel size in maize (*Zea mays*)

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Abstract

Background Kernel size are important agronomic traits for grain yield in maize. The objective of this study is to map QTLs for kernel size, fine map a stable QTL qKL-2 and predict candidate genes for kernel length in maize. A total 199 F2:3 lines were obtained from selfing F2 individuals from the cross SG5/SG7. In this study, a high density genetic linkage map with 3305 bin-markers spanning a total length of 2236.66 cM on 10 chromosomes was applied for primary QTL mapping. Composite interval mapping (CIM) method was used for detecting QTLs in three environments of F2 and F2:3 populations.

Results The result showed that a total ten QTLs for kernel size were detected, among which five QTLs for kernel length (KL) and five QTLs for kernel width (KW). Two stable QTLs qKW-1 and qKL-2 were mapped in all three environments. In order to validate and fine map qKL-2, near isogenic lines (NILs) were developed by continuous backcross between SG5 as the donor parent and SG7 as the recurrent parent. Marker assisted selection was conducted from BC2F1 generation with molecular markers near qKL-2. Secondary linkage map with six markers around the objective region was developed for fine mapping qKL-2. Finally, qKL-2 was mapped in a 1.95Mb physical interval on maize chromosome 9 by blasting with Zea_Mays_B73 v4 genome. The results were confirmed with selected overlapping recombinant chromosomes. A total 11 out of 40 protein coding genes in the identified interval differentially expressed after conducting transcriptomic analysis between the two parents. GRMZM2G006080, which encodes receptor-like protein kinase FERONIA was predicted as candidate gene to control kernel size.

Conclusions A total ten QTLs for kernel size were identified. Two stable QTLs qKL-2 and qKW-2 were mapped in three environments. Major QTL qKL-2 for KL was validated and fine mapped in a 1.95Mb physical interval. GRMZM2G006080 was predicted as candidate gene for qKL-2 to control kernel length. The work will not only help to understand the mechanisms that control kernel size of maize, but also provide new gene for marker-assisted selection in further studies.

Background

Maize is one of the most important crops served as food or animal feed[1] and plays a special role in sustaining food security[2]. To obtain high grain yield is one of the most important objectives for maize breeding. Yield traits are complex quantitative traits controlled by multiple genes. Kernel size traits are considered as important yield traits in improving grain yield process[3]. Kernel size which are thought of important component of grain yield and are consisted of kernel length, width and thickness and determines kernel weight [4]. Kernel size traits, especially kernel length have been revealed to significantly correlate with grain yield [5]. Therefore, improving kernel size have great meaning in maize breeding.

During the twentieth century, a lot of maize grain yield traits improvement works from phenotypic level were conducted [6]. However, it is difficult to illustrate the specific reasons that yield formation affected from only phenotypic level. Along with the first genetic linkage map of maize published in 1986 [7], Molecular markers based on polymerase chain reaction (PCR) technology have greatly developed and applied for constructing genetic maps. Then, more and more QTLs controlling important agronomic traits in maize were detected by analyzing phenotypic value and genotypic value based on constructed genetic maps. These identified QTLs were distributed on all 10 maize chromosomes [8]. Till now, more than 150 QTLs for kernel size or weight have

been identified by using different populations in maize (Gramene QTL database). Many QTL mapping or fine mapping works for kernel size or weight have been carried out recent years [2, 9, 10].

Great achievements in QTL mapping or isolating underlying genes for kernel size have made in many species such as rice [11–15], *Arabidopsis thaliana* [16, 17], soybean [18, 19], wheat [20, 21], especially in rice, genes control kernel size or weight such as GS3 [22], GS5 [11], qGL3 [23], GW2 [13], GW8 [24], GS2 [25], qGW7/GL7 [26] have been successfully cloned. Compared with studies on kernel size in species mentioned above, studies on identifying and cloning kernel size related genes has lagged behind in maize. To a certain extent, the reason for this might be low QTL mapping accuracy and low QTL mapping resolution based on genetic linkage maps with low marker density [27]. Along with the development in the next-generation sequencing (NGS) technologies and the continuous declining cost of genotyping, it is possible to develop high-density genetic linkage maps in maize. In addition, it is critical important that QTLs should be validated and fine mapped for applying in further marker assisted breeding process. Near isogenic line (NIL) is one of the most widely accepted populations commonly used in QTL fine mapping. NILs have been successfully used in confirming and fine-mapping QTLs in many species, such as rice [28–30], wheat [31, 32]. In maize, Nie et al. 2019 fine mapped a major QTL qkrnw4 associated with kernel row number by the use of a near-isogenic line (NIL) [33]. Gao et al. 2019 fine mapped qLRI4 conferring leaf rolling index by using NIL populations [34]. Yang et al. 2018 fine mapped a major QTL qkc7.03 to a 416.27 kb physical interval for kernel cracking with NILs developed [35].

Based on above, the purposes of this study were. 1) to map QTLs for kernel size in three environments by using F_2 and $F_{2:3}$ populations from the same cross SG5/SG7. 2) to validate and fine map the identified major QTL qKL-2 by using BC_3F_1 NILs. 3) RNA-seq technology was applied to reveal DEGs between SG5 and SG7 and overlay these DEGs on to the fine mapped physical intervals of qKL-2 to predict candidate genes responsible for kernel length.

Results

Segregation population development and phenotyping analysis

Phenotypic values of F_2 population were collected for two kernel size traits i.e., kernel length (KL) and kernel width (KW) in 2016 while phenotypic values of $F_{2:3}$ populations were collected for the two traits in 2018 and 2019. Phenotypic mean values of KL and KW in F_2 and $F_{2:3}$ were shown in Table 1. The difference of KL between SG5 and SG7 is extremely significant ($P < 0.01$, Figure 3(a), Figure 3(b)). The phenotypic value of SG5 and SG7 were significantly different in both KL and KW traits. The two observed kernel size traits both displayed as bell shaped normal distribution (Supplemental Fig.1). Pearson's correlation coefficients between KL and KW is 0.20, 0.25 and 0.24 in F_2 -2016, $F_{2:3}$ -2018 and $F_{2:3}$ -2019, respectively. The correlation coefficients between KL and KW were all significant in three environments.

QTL mapping

CIM procedure was applied for mapping QTLs conferring KL and KW. Ten QTLs were mapped for KL and KW in F_2 and $F_{2:3}$ populations. In F_2 population, two KL QTLs were mapped and both located on maize chromosome 9, three KW QTLs were mapped and located on maize chromosomes 3 and 8. In $F_{2:3}$ -2018 population, four KL QTLs were mapped and located on maize chromosome 3, 7, and 9, three KW QTLs were mapped and located on

maize chromosomes 3 and 8. In $F_{2:3}$ -2019 population, three KL QTLs were mapped on maize chromosome 7, and 9, three KW QTLs were mapped and located on maize chromosomes 3 and 8. Manhattan plots of QTL mapping results were shown in Fig. 1. The physical intervals for all ten detected QTLs spanned from 2.30 to 14.35 Mb by comparison to reference genome (B73 RefGen_v3). The phenotypic variation explained by each QTL ranged from 8.4 to 23.0% in a trait, with means of 14.25, 14.03 and 13.67% for traits KL in F_2 -2016, $F_{2:3}$ -2018 and $F_{2:3}$ -2019 respectively while means of 14.63, 12.97 and 10.83% for traits KW in three environments. The LOD scores range from 4.0 ($qKL-7$) to 9.5($qKW-1$). Information of QTL identified are summarized in Table 2. The results showed that, $qKL-2$ for KL and $qKW-1$ were both detected in all three environments (highlighted with green color circle in Fig. 2). Major QTLs $qKW-2$, $qKL-7$, $qKW-3$ and $qKL-10$ were detected repeatedly in two environments. (highlighted with blue color circle in Fig. 2). To sum up, QTLs $qKL-2$ and $qKW-1$ detected in this study are considered to be stable QTLs (Table 2). However, results above are primary mapping results, which need to be validated and fine mapped in further study.

NILs development and $qKL-2$ fine mapping

In 2017 to 2019, a total of 998 BC_3F_1 NILs of $qKL-2$ with *SG7* background were developed by continuous backcross method. A secondary linkage map with six separated markers around $qKL-2$ was generated. The sequences of the associated markers were listed Supplemental Table 1. The total length of the map was 43.35cM and the genetic distances between each two adjacent markers were 16.75, 8.39, 0.80, 5.67 and 11.74 cM, respectively. The physical positions of the six markers were 115.23, 130.51, 133.34, 135.29, 139.75 and 153.88Mb on chromosome 9 by blasting maize B73 RefGen_v4(Figure 3(c)). Major QTL $qKL-2$ was detected on the secondary linkage map of NILs based on CIM method in QTL Cartographer v2.5. The peak of LOD value indicating that $qKL-2$ was most likely located between SSR3 and SSR4 (Figure 3 (c)). The additive effect of $qKL-2$ was 0.97mm and the phenotypic variation was 16.0%. To confirm the narrowed $qKL-2$ interval, Five kind of recombinant types named Class 1 to Class 5 were selected from 998 BC_3F_1 individuals, and the progeny test of homozygous segregants indicated the interval to a 1.95-Mb region flanked by the markers SSR3 and SSR4 (table 3). There are significant difference between phenotypic values of the two set of recombinant types Class 2,3 and Class 1, 4, 5(Figure 3 (d)). The loci of SSR3 and SSR4 are homozygous in Class 2,3 while heterozygous in Class 1, 4, 5. The results that $qKL-2$ located in SSR3 to SSR4 interval also supported by selected overlapping recombinant chromosomes.

Candidate genes prediction for $qKL-2$

RNA-seq procedure was conducted on an Illumina NovaSeq instrument with 18 RNA samples from grains in different developmental stages of plants. The 1.95-Mb physical intervals of $qKL-2$, encompass 40 protein coding genes, (figure 3(e)). After DEGs analysis, a total of 11 protein coding genes left in the physical intervals (Table 4). Previous studies indicated that FERONIA receptor kinase controls seed size in *Arabidopsis thaliana*[41]. GRMZM2G006080 encodes receptor-like protein kinase FERONIA and was predicted as candidate gene of $qKL-2$ which most likely responsible for kernel length.

Discussion

Kernel size traits which controlled by multiple genes are important components of grain yield in maize. Grain yield was influenced significantly by kernel size, especially kernel length [4, 5]. Stable QTLs are of great

significance for marker-assisted breeding while false positive QTLs are no use. Normally, two steps i.e. primary mapping and fine mapping are needed for QTL analysis unless experiments were conducted in multiple environments with as many as sample size and marker numbers. In this study, primary mapping were carried out in two environments, three kernel size QTLs qKL-2 and qKW-1 detected in all three environments were stable. The two QTLs could be benefit for further marker assisted breeding. Advanced population was developed by continuous backcross with markers assisted selection for validating and fine mapping qKL-2. Finally, qKL-2 was mapped in a 1.95-Mb interval on maize chromosome 9, the results was confirmed with overlapping recombinant chromosomes. It is of critical importance that the less genes the better in target QTL interval for map based cloning. In this study, RNA-seq technology was applied for transcriptomic analyzing DEGs between SG5 and SG7 grains in different developmental stages. DEGs identified were overlaid on to the qKL-2 intervals. After DEGs analysis, only 11 protein coding genes left in the QTLs qKL-2 intervals (Table 4). The potential functional genes in QTLs physical intervals decreased significantly after DEGs analysis. Further comparative genomics analysis was applied for predicting candidate genes. The evidence on studies of rice or arabidopsis thaliana showed that kernel size was regulated by multiple signaling pathways mainly include, ubiquitin-proteasome degradation [42], transcription factor pathway, phytohormone signaling pathway and G protein independent pathway. Yu et al. 2014 conclude that receptor kinase FERONIA involved in a signaling pathway to negatively regulate the elongation of integument cells, leading to control of the seed size in Arabidopsis thaliana [41]. Therefore, GRMZM2G006080, which encodes receptor-like protein kinase FERONIA was predicted as candidate gene to control kernel size. The predicted candidate gene will be not only helpful for underlying genetic mechanism for kernel size, but also provide basis for improving kernel size traits in maize.

Conclusions

In this study, a total ten QTLs for kernel size were detected, among which five QTLs for kernel length (KL) and five QTLs for kernel width (KW). Two stable QTLs qKL-2 and qKW-2 were mapped in three environments. Major QTL qKL-2 for KL was validated and fine mapped in a 1.95 Mb physical interval on maize chromosome 9 by blasting with Zea_Mays_B73 v4 genome. The results were confirmed with selected overlapping recombinant chromosomes. GRMZM2G006080, which encodes receptor-like protein kinase FERONIA was predicted as candidate gene to control kernel length.

Methods

Primary population development and phenotypic evaluation

Maize inbred lines SG5 and SG7 were obtained from Institute of Grain and Oil, Liupanshui Academy of Agricultural Sciences, Liupanshui, China. F₁ hybrid seeds were obtained from crossing two maize inbred lines SG5 and SG7 in Liupanshui, Guizhou province of China in 2013. The hybrid seeds were planted in the same location in 2014 summer. A total of 199 F₂ individuals were developed by planting F₁ seeds at the Panxian Maize Breeding Station in Sanya, Hainan Island of China in winter 2014. Next, a F_{2:3} segregation population with 199 lines was developed from selfing each F₂ individuals. A total of 199 lines of the F_{2:3} population was planted in field with a randomized block design of three replications, single-row plot with row spacing 50cm, each plot 15 plants with plants spacing 35cm. Kernel size traits included kernel length(KL) and kernel width (KW) were investigated by using electronic digital calipers. In F_{2:3}, Kernel size traits of 8 ears in 8 single plants located in

the middle of each plot were chosen for evaluating phenotypic value after harvesting and drying naturally step. In F_2 , phenotypic values of KL and KW were estimated by mean values from three replications of 10 kernels randomly selected from middle parts of the ear. In $F_{2:3}$, KL and KW were estimated by mean values from three replications of 10 kernels randomly selected from bulked kernels of middle parts of 8 ears.

QTL mapping

Methods of extracting genomic DNA, sequencing genotype, grouping sequence data, identifying single nucleotide polymorphisms (SNPs) and constructing high-density linkage map results were exhibited in our previous study [36]. *Forward regression model of CIM* method in QTL Cartographer v2.5 was applied for QTL mapping with walking speed was 1 cM. The logarithm of odds (LOD) value for detected traits was set up by 1,000 times permutations results. The LOD significance threshold for declaring QTL was 3.86 for CIM based upon the permutation analysis. QTL statistics were also reported for those in which the LOD score exceeded 2.5. LOD peaks were used for determining the position of a significant QTL on chromosomes. The additive effect value of a QTL is positive indicate that the increase in phenotypic value for a trait is provided by SG5 alleles while negative indicate the decrease in phenotypic value for a trait is provided by SG7 alleles. MapChart 2.32 software [37] was used for drawing the graphic of QTLs positions on 10 chromosomes of maize. Physical intervals of the QTLs mapped in F_2 and $F_{2:3}$ populations respectively were compared together and QTLs which have identical physical intervals in both two populations will be regarded as stable QTLs. The QTL mapping software carried out here also evaluates additive, dominance effect and the phenotypic variation by a QTL identified.

NILs development and $qKL-2$ fine mapping

NILs population was developed by using continuous backcross method for $qKL-2$ locus. Marker assisted selection started from BC_2F_1 generation with SSR molecular markers near $qKL-2$ between SG5 as the donor parent and SG7 as the recurrent parent. Primers of SSR markers were all selected from primers developed by Xu et al. 2013 based on resequencing maize genome results [38]. Phenotypic value for each of BC_3F_1 individual was investigated the same to method conducted in F_2 population, which used for primary mapping in this study. Young healthy leaves from each of the 998 BC_3F_1 individuals were collected and frozen in liquid nitrogen, and then transferred to a -80°C freezer. Genomic DNA of all individuals were extracted following the manufacturer's protocols with the Plant Genomic DNA Kit (TIANGEN, Beijing, China). DNA degradation and contamination were monitored on 1% agarose gels. DNA purity was checked using the NanoPhotometer R spectrophotometer (IMPLEN, CA, USA). DNA concentration was measured using Qubit R DNA Assay Kit in Qubit R 2.0 Fluorometer (Life Technologies, CA, USA). Secondary linkage map with selected markers around $qKL-2$ was generated by using JoinMap 3.0 software [39] with a logarithm of the odds (LOD) critical value 10.0 as the threshold. CIM method in QTL Cartographer v2.5 was applied for QTL mapping with walking speed 1 cM.

Sample preparation, RNA isolation and data analysis

The two parents SG5 and SG7 were planted in the field. Ear shoots were covered by ear shoot bags before corn silk grow out. SG5 and SG7 plants in the middle of the plot were chosen for selfing at the same day at the stage of the corn silk length became about 5cm. Grains of the selfing parents' plants were sampled at the different developmental stages (5 days, 10 days, and 15 days after selfing) with each sample of three biological

replicates in parallel. All collected samples were immediately frozen in liquid nitrogen and transferred to -80°C storage environment. Methods of extracting total RNA, checking RNA purity, measuring RNA concentration, assessing RNA integrity and performing RNA-seq were exhibited in our previous study[40]. A total of 18 collected samples were sequenced at the Illumina NovaSeq platform. Softwares for building reference genome index, aligning paired-end clean reads to the reference genome, counting reads numbers mapped to each gene, calculating expected number of fragments per kilobase of transcript sequence per millions base pairs (FPKM) and analyzing differential expression between SG5 and SG7 were exhibited in our previous study[40].

Candidate genes prediction for $qKL-2$

In this study, RNA-seq data was used for mining differentially expressed genes (DEGs) between SG5 and SG7. Pair-wise comparison of transcriptomes between SG5 and SG7 was conducted for detecting DEGs. The DEGs obtained were overlaid on to physical intervals of $qKL-2$ to predict candidate genes for kernel length in maize. The detected DEGs were annotated on the basis of database for maize gene annotation accessible at MaizeGDB website <http://www.maizegdb.org>.

Abbreviations

BLAST Basic local alignment search tool

CIM Composite interval mapping

LOD Logarithm of odds

QTL Quantitative trait loci

NIL Near isogenic line

SNP Single nucleotide polymorphisms

DEG Differentially expressed gene

Declarations

Ethics approval and consent to participate:

Not applicable.

Consent for publication:

Not applicable.

Availability of data and materials:

Data supporting the current study can be obtained by contacting the corresponding author (chfsu2008@163.com). Seed of SG5 and SG7 is available from the authors upon request.

Competing Interests

We declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted.

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Author Contributions

GW and YZ have finished in developing F_2 , $F_{2:3}$ and BC_1F_3 population. ZZ, XZ, MJ and MC have finished phenotypes investigation. CS have finished genotyping of $F_{2:3}$ progeny, analyzing the data, and drafted the manuscript. All authors read and approved the final manuscript.

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Tables

Table 1. Descriptive statistics of traits in the F₂ and F_{2:3} mapping population of maize derived from the cross of SG5 and SG7.

| Generations | Trait | SG5(mm) | SG7(mm) | Min(mm) | Max(mm) | Mean(mm) | Std.Dev.(mm) |
|------------------------|-------|---------|---------|---------|---------|----------|--------------|
| F ₂ | KL | 9.93 | 8.99 | 8.07 | 12.87 | 10.42 | 0.85 |
| | KW | 8.07 | 11.17 | 8.03 | 11.80 | 9.98 | 0.78 |
| F _{2:3} -2018 | KL | 9.93 | 8.99 | 8.49 | 13.21 | 10.34 | 0.74 |
| | KW | 8.07 | 11.17 | 8.24 | 12.12 | 10.07 | 0.74 |
| F _{2:3} -2019 | KL | 9.93 | 8.99 | 8.40 | 13.35 | 10.34 | 0.75 |
| | KW | 8.07 | 11.17 | 8.28 | 12.22 | 10.08 | 0.73 |

Table 2. QTL identified for KL and KW traits of maize using high-density SNP bin-map from composite interval mapping (CIM). The logarithm of odds (LOD) value 3.86 for detected traits was set up by 1,000 times permutations results (in blod). QTL statistics were also reported for those in which the LOD score exceeded 2.5 but less than 3.86 (in no blod).

| Environments | QTL | Chr | Flanking markers | Positions (Mb) | Interval (Mb) | Physical length (Mb) | LOD | ADD ^a | DOM ^b | R ² (%) |
|---------------------------------|---------------|-----|---------------------------|----------------|----------------------------|----------------------|------------|------------------|------------------|--------------------|
| Kernel length (KL) trait | | | | | | | | | | |
| F ₂ -2016 | qKL-1 | 9 | mk3093- mk3100 | 126.05 | 124.90- 127.55 | 2.65 | 8.4 | 0.34 | 0.18 | 14.8 |
| | qKL-2 | 9 | mk3106- mk3114 | 134.65 | 133.20- 135.75 | 2.55 | 7.7 | 0.26 | 0.24 | 13.7 |
| | qKL-3 | 3 | mk1343- mk1350 | 217.85 | 215.90- 218.1 | 2.20 | 2.9 | -0.13 | -0.17 | 4.7 |
| | qKL-4 | 2 | mk624- mk638 | 2.75 | 0.10-3.55 | 3.45 | 2.5 | 0.06 | 0.254 | 4.2 |
| | qKL-5 | 10 | mk3228- mk3236 | 105.05 | 96.45-109.2 | 12.75 | 3.8 | -0.19 | -0.14 | 6.4 |
| F _{2:3} -2018 | qKL-2 | 9 | mk3105- mk3110 | 134.65 | 131.55- 134.75 | 3.20 | 8.1 | 0.23 | 0.25 | 15.3 |
| | qKL-4 | 2 | Mk624- mk634 | 2.85 | 0.10-3.00 | 2.90 | 3.1 | -0.02 | 0.31 | 5.4 |
| | qKL-6 | 3 | mk1145- mk1167 | 115.20 | 103.65- 117.85 | 14.2 | 4.2 | -0.38 | 0.17 | 12.1 |
| | qKL-7 | 7 | mk2510- mk2525 | 143.00 | 137.30- 145.70 | 8.40 | 5.1 | -0.40 | 0.32 | 13.4 |
| | qKL-8 | 7 | mk2618- mk2622 | 174.6. | 174.35- 174.90 | 0.55 | 3.5 | -0.28 | 0.04 | 6.5 |
| | qKL-9 | 8 | mk2689- mk2701 | 21.85 | 21.60-33.80 | 12.20 | 3.1 | -0.30 | 0.20 | 8.0 |
| | qKL-10 | 9 | mk3077- mk3084 | 115.15 | 113.65- 120.70 | 7.05 | 8.1 | 0.30 | 0.19 | 15.3 |
| F _{2:3} -2019 | qKL-2 | 9 | mk3100- mk3104 | 128.0. | 127.55- -130.05 | 2.50 | 6.9 | 0.27 | 0.17 | 13.4 |
| | qKL-4 | 2 | mk624- mk637 | 2.85 | 0.10-3.45 | 3.35 | 2.6 | 0.00 | 0.27 | 4.5 |
| | qKL-6 | 3 | mk1158- mk1167 | 115.2. | 114.80- 117.85 | 3.05 | 3.9 | -0.37 | 0.19 | 11.3 |
| | qKL-7 | 7 | mk2517- mk2525 | 143.00 | 141.25- 145.70 | 4.45 | 4.0 | 0.40 | 0.34 | 13.0 |
| | qKL-8 | 7 | mk2617- mk2622 | 174.60 | 174.05- 174.90 | 0.85 | 3.7 | -0.26 | -0.01 | 5.9 |
| | qKL-10 | 9 | mk3077- mk3084 | 115.15 | 113.65- 120.70 | 7.05 | 7.6 | 0.28 | 0.20 | 14.6 |
| | qKL-11 | 5 | mk2183- mk2188 | 215.30 | 214.55- 215.55 | 1.00 | 2.6 | 0.25 | -0.26 | 5.9 |
| | qKL-12 | 6 | mk2298- mk2303 | 149.80 | 143.05- 157.50 | 14.45 | 2.5 | 0.09 | 0.24 | 4.4 |
| Kernel width (KW) trait | | | | | | | | | | |
| F ₂ -2016 | qKW-1 | 3 | mk1042- mk1060 | 30.85 | 30.20-44.55 | 14.35 | 9.5 | -0.54 | 0.17 | 23.0 |
| | qKW-2 | 8 | mk2806- mk2814 | 148.95 | 144.55- 151.00 | 6.45 | 5.8 | -0.34 | -0.02 | 10.0 |
| | qKW-3 | 8 | mk2814- mk2820 | 152.25 | 151.00- 157.95 | 6.95 | 5.9 | -0.35 | 0.00 | 10.9 |
| | qKW-4 | 1 | mk577- mk603 | 292.20 | 288.45- 295.55 | 7.10 | 3.1 | -0.29 | 0.09 | 7.2 |
| | qKW-5 | 2 | mk667- mk673 | 17.05 | 13.60-17.55 | 3.95 | 3.4 | -0.23 | -0.07 | 4.5 |
| | qKW-6 | 2 | mk676- mk686 | 24.05 | 19.55-24.35 | 4.80 | 3.7 | -0.19 | -0.13 | 6.1 |
| | qKW-7 | 10 | mk3284- mk3294 | 145.50 | 144.70- 146.95 | 2.25 | 3.0 | -0.15 | 0.33 | 4.9 |
| F _{2:3} -2018 | qKW-1 | 3 | mk1040- mk1047 | 30.85 | 29.30-32.75 | 3.45 | 5.8 | -0.37 | -0.04 | 9.9 |
| | qKW-2 | 8 | mk2811- mk2814 | 148.95 | 147.80- 151.00 | 3.20 | 5.8 | -0.43 | 0.13 | 14.5 |

| | | | | | | | | | | |
|------------------------|----------------------|----------|---------------------------|---------------|---------------------------|-------------|------------|--------------|--------------|-------------|
| | <i>qKW-5</i> | 2 | mk667- mk676 | 16.70 | 13.60-19.55 | 5.95 | 3.2 | -0.12 | -0.20 | 5.3 |
| | <i>qKW-8</i> | 8 | mk2799- 2808 | 141.80 | 140.30- 145.60 | 5.30 | 6.2 | -0.43 | 0.11 | 14.5 |
| | <i>qKW-9</i> | 2 | mk657- mk662 | 9.70 | 9.30-11.30 | 2.00 | 3.8 | -0.06 | -0.29 | 6.2 |
| F _{2,3} -2019 | <i>qKW-1</i> | 3 | mk1035- mk1042 | 28.95 | 27.10-30.20 | 3.10 | 5.5 | -0.47 | 0.19 | 14.5 |
| | <i>qKW-3</i> | 8 | mk2814- mk2817 | 152.25 | 151.00- 153.30 | 2.30 | 5.5 | -0.33 | -0.01 | 9.6 |
| | <i>qKW-4</i> | 1 | mk579- mk591 | 292.55 | 289.45- 293.25 | 3.80 | 2.5 | -0.23 | 0.05 | 5.0 |
| | <i>qKW-6</i> | 2 | mk676- mk686 | 23.75 | 19.55-24.35 | 4.80 | 3.8 | -0.17 | -0.14 | 6.2 |
| | <i>qKW-10</i> | 8 | mk2826- mk2835 | 163.25 | 160.70- 164.95 | 4.25 | 5.0 | -0.24 | -0.14 | 8.4 |

^a Estimated additive effect

^b Estimated dominance effect

Table 3. Statistical analysis of phenotypic values from different kinds of recombinant types of NILs around the *qKL-2* region

| | No.of | Grain length/mm |
|---------|--------------|-----------------|
| | Recombinants | |
| class 1 | 28 | 10.85±0.14 |
| class 2 | 33 | 9.88±0.13 |
| class 3 | 3 | 9.99±0.16 |
| class 4 | 20 | 10.71±0.19 |
| class 5 | 47 | 10.89±0.11 |

Table 4. Differentially expressed genes out of 40 protein coding genes in 1.95-Mb physical interval on chromosome 9 and candidate gene predicted for *qKL-2*.

| geneID[B73 RefGen_v3] | start(bp) | end(bp) | length | Annotation | LogFC ^d or RCP1/RCP2 ^e | | |
|--------------------------|-----------|-----------|--------|--|--|---------|--------|
| | | | | | Day5 ^f | Day10 | Day15 |
| GRMZM2G099101 | 132128943 | 132133712 | 2349 | Endoglucanase 9 | 0.36 | 0.42 | 1.66 |
| GRMZM5G899188 | 132973437 | 132974250 | 814 | 17.0 kDa class II heat shock protein | 2.03 | 0.98 | 3.23 |
| GRMZM2G398288 | 131627588 | 131635485 | 4521 | Phospholipid- transporting ATPase 1 | -0.40 ^g | 0.36 | -0.22 |
| GRMZM2G027437 | 131778230 | 131786437 | 1277 | Receptor-like protein kinase FERONIA | -0.55 | -0.45 | -0.79 |
| GRMZM2G006080 | 131829772 | 131832943 | 3172 | | 0.41 | -0.09 | -0.04 |
| GRMZM2G309327 | 131924112 | 131925141 | 1030 | Probable calcium- binding protein | -1.87 | -2.20 | -1.41 |
| GRMZM2G159500 | 133066105 | 133068297 | 2048 | NAC domain- containing protein | -0.76 | -0.22 | -0.21 |
| GRMZM2G102382 | 133137426 | 133140041 | 1413 | Thioredoxin- like 1-2, chloroplastic | 0.85 | 0.95 | 1.01 |
| GRMZM2G102657 | 132797892 | 132800350 | 2053 | 17.0 kDa class II heat shock protein | 0/9.54 | 0/18.55 | 0/7.55 |
| GRMZM2G404249 | 132803660 | 132804501 | 842 | | 1.70 | 2.29 | 1.27 |
| GRMZM5G875954 | 132964847 | 132972815 | 1206 | Selenium- binding protein 2 | -5.07 | -3.20 | -1.20 |

d: Log 2 ratio, number of folds the gene is differentially expressed in RNA-seq;

e: different of readcounts between P₁ and P₂;

f: day5-day10-day15 indicate grain samples collected after selfing 5 days, 10 days and 15 days between the two parents SG5 and SG7.

g: Positive sign indicates gene transcript expressed high in SG5 while negative sign indicates gene transcript expressed high in SG7

Figures

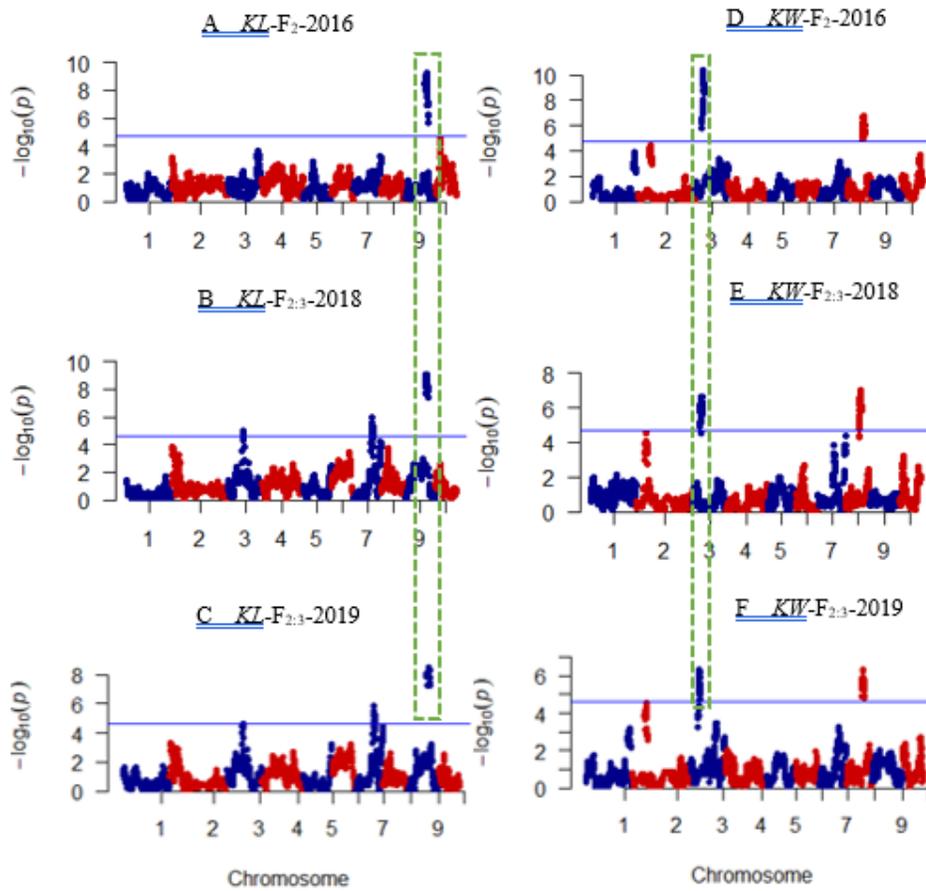


Figure 1

Plots of test statistic $-\log_{10}(p)$ against genome location for KL and KW traits in maize using the CIM method. The horizontal blue line of each panel is the critical value of the test statistic generated from 1,000 permuted samples. Figure A, B and C indicate KL mapping results in three environments while Figure D, E and F indicate KW mapping results in three environments. Dotted rectangle with green color indicate these QTLs were mapped repeatedly in all three environments.

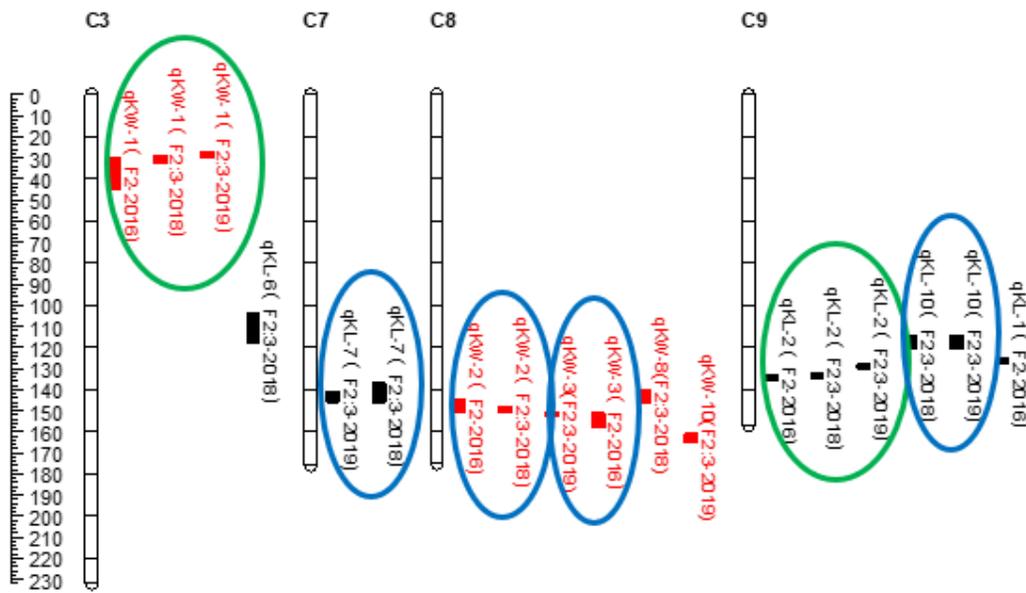


Figure 2

QTL locations for KL and KW traits studied in the F2 and F2:3 populations from cross SG5/SG7. QTLs were represented in different colors for kernel size traits including red for KW (kernel width, mm), black for KL (kernel length, mm) on chromosomes C3, C7, C8 and C9. QTLs represented by bars are shown on the right of the linkage groups, close to their corresponding markers. Supported intervals for each QTL are indicated by the length of vertical bars. The QTLs circled in green were stably detected in three environments while QTLs circled in blue were repeatedly detected in two environments.

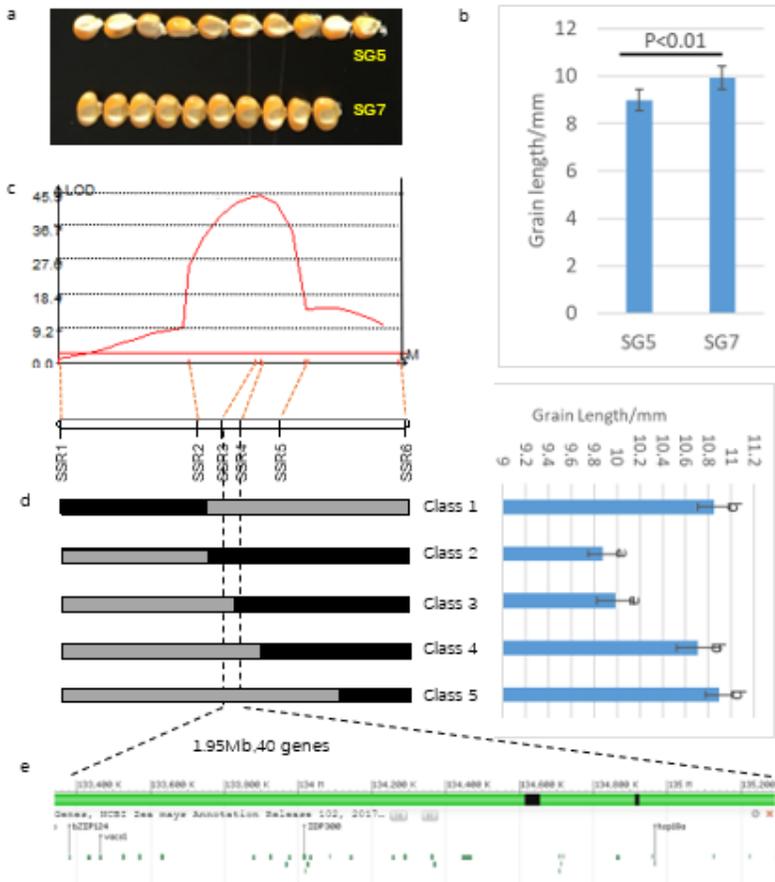


Figure 3

a. Grain length difference between SG5 and SG7. b. significance test of difference between SG5 and SG7, $P < 0.01$ means that difference was extremely significant between SG5 and SG7; c. LOD profile of qKL-2, which was identified in the BC3F1 populations. d. qKL-2 was mapped to a 1.95-Mb genomic DNA region between the markers SSR3 and SSR4 by using 998 BC3F1 plants. Class 1 to Class 5 are shown for the recombinant plants with different recombination types. Graphical genotypes of recombinant lines and their grain length separated in BC3F1. Black bars and grey bars represent the chromosomal segments for the homozygous SG7 and heterozygous alleles, respectively. Different letters indicate significant difference at 0.05 level. e. physical positions of the forty protein coding genes in the mapped 1.95 Mb region (B73 RefGen_v4).

Supplementary Files

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- [SupplementalFig.1.docx](#)
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