Fine mapping and validation of a major QTL for grain weight on chromosome 5B in bread wheat

Dehui Zhao1,2 · Li Yang1 · Dan Liu1 · Jianqi Zeng1 · Shuanghe Cao1 · Xianchun Xia1 · Jun Yan3 · Xiyue Song2 · Zhonghu He1,4 · Yong Zhang1

Received: 7 April 2021 / Accepted: 23 July 2021 / Published online: 29 July 2021
© The Author(s), under exclusive licence to Springer-Verlag GmbH Germany, part of Springer Nature 2021

Abstract

Key message A major QTL QTgw.caas-5B for thousand grain weight in wheat was fine mapped on chromosome 5B, and TraesCS5B02G044800 was predicted to be the candidate gene.

Abstract Thousand grain weight (TGW), determined by grain length and width, and is an important yield component in wheat; understanding of the underlying genes and molecular mechanisms remains limited. A stable QTL QTgw.caas-5B for TGW was identified previously in a RIL population developed from a cross between Zhongmai 871 (ZM871) and a sister line Zhongmai 895 (ZM895), and the aim of this study was to perform fine mapping and validate the genetic effect of the QTL. It was delimited to an interval of approximately 2.0 Mb flanked by markers Kasp_5B29 and Kasp_5B31 (49.6–51.6 Mb) using 12 heterozygous recombinant plants obtained by selfing a residual BC1F6 line selected from the ZM871/ZM895/ZM871 population. A candidate gene was predicted following sequencing and differential expression analyses. Marker Kasp_5B_Tgw based on a SNP in TraesCS5B02G044800, the QTgw.caas-5B candidate, was developed and validated in a diversity panel of 166 cultivars. The precise mapping of QTgw.caas-5B laid a foundation for cloning of a predicted causal gene and provides a molecular marker for improving grain yield in wheat.

Introduction

Wheat is one of the most important food crops in the world, providing approximately 20% of the calories and 25% of the protein for humans (FAO 2017, http://www.fao.org/faostat/en/). Although significant progress has already been made on yield improvement during the last 60 years, with a genetic gain of 0.7–1.0% annually (Fischer and Edmeades 2010; Gao et al. 2017), it is estimated that yield must still increase by more than 60% to meet predicted growth in the world population by 2050, despite the restricting effects of climate change and the declining area of available land due to urbanization and degradation (Langridge 2013). Improved yield potential is, therefore, still a major breeding objective. Identification and mining of genetic loci for grain yield will provide genetic resources and tools to improve yield potential.

Yield is a complex quantitative trait determined by thousand grain weight (TGW), grain number per spike and spike number per unit area, with TGW having the highest heritability among three components (Simmonds et al. 2014, 2016; Chen et al. 2020; Yang et al. 2020). TGW is determined by grain size and grain filling characteristics. Grain size can be divided into components grain length (GL), grain width (GW) and grain thickness (Kuchel et al. 2007; Simmonds et al. 2014; Yang et al. 2020). Numerous genes for grain weight have been cloned in rice (Li and Li 2016; Li et al. 2019b), and many genes associated with grain weight in wheat were cloned by comparative genomics (Su et al. 2015).
Zhongmai 895 (hereafter ZM895), with a current production of 0.7 million ha annually is a leading cultivar in the Yellow and Huai River Valleys Winter Wheat Region of China. ZM895 and Zhongmai 871 (hereafter ZM871), developed by pedigree selection and bulked as fixed lines at F$_5$, are sister lines that can be traced back to a single F$_2$ plant of cross Zhoumai 16/Liken 4. The detailed information of ZM895 and ZM871 was described in a previous study (Yang et al. 2020). One residual heterozygous line (L2925) within the marker interval of QTgw.caas-5B was selected from BC$_1$F$_6$ generation of the HRL2925 were evaluated by genotyping two 5B+ and two 5B− homozygous lines generated from two kinds of homozygous plants, respectively, using the wheat 50 K SNP array developed in collaboration with the Capital-Bio, Beijing, China (https://www.capitalbiotech.com/). Twenty 5B+ and 20 5B− homozygous lines were used to measure TGW, TL and GW at different growth stages and for RNA-sequencing. In addition, 52–77 NILs from each of RL1 to RL12 were identified using QTgw.caas-5B-flanking markers to narrow the region of candidate genes (Fig. 1d; Table 2). A diverse panel of 166 cultivars (Li et al. 2019a) was used to validate the effects of QTgw.caas-5B.

**Field trials and trait measurement**

The progeny from HRL2925 were sown in ten 3.0 m rows spaced 0.3 m apart with 30 seeds per row at Xinxiang (Henan province) during the 2017–2018 cropping season. Twenty homozygous lines with 5B+ and 20 with 5B− selected from 119 self-pollinated homozygous plants of HRL2925 were evaluated at Xinxiang and Anyang (Henan province) during the 2018–2019 cropping season. The lines were grown as plots in randomized complete blocks with three replications. Each plot comprised two 1.0 m rows spaced 0.3 m apart, with 30 seeds per row. Twelve segregating populations derived from the recombinants were sown in plots of seven 3.0 m rows spaced 0.3 m apart with 30 seeds per row at Xinxiang (Henan province) during the 2018–2019 cropping season. The panel of 166 cultivars was sown in three 1.5 m rows spaced 0.2 m apart in 50 seeds each row with three replications at Suixi (Anhui province) during the 2012–2013, 2013–2014 and 2014–2015 cropping seasons, at Anyang (Henan province) during the 2012–2013 and 2013–2014 cropping seasons, and at Shijiazhuang (Hebei province) during the 2014–2015 cropping season.

Wanshen SC-G seed detector (Hangzhou Wanshen Detection Technology Co., Ltd) was used to record TGW, GL and GW. Thirty random spikes were harvested from each plot of all 20 homozygous individuals in the contrasting 5B+ and 5B− groups to measure TGW, TL and GW. The same parameters were measured on the 119 homozygous plants from HRL2925 were measured on grains from 6–10 spikes of each plant and 52–77 homozygous progenies from each of RL1–RL12. For the diversity panel, TGW was determined by weighing 500 grains, and TL and GW were measured on 20 random grains from each plot to obtain mean length and width values, respectively (Li et al. 2019a).

---

**Materials and methods**

**Plant materials**

Zhongmai 895 (hereafter ZM895), with a current production area around 0.7 million ha annually is a leading cultivar in the Yellow and Huai River Valleys Winter Wheat Region of China. ZM895 and Zhongmai 871 (hereafter ZM871), developed by pedigree selection and bulked as fixed lines at F$_5$, are sister lines that can be traced back to a single F$_2$ plant of cross Zhoumai 16/Liken 4. The detailed information of ZM895 and ZM871 was described in a previous study (Yang et al. 2020). One residual heterozygous line (L2925) within the marker interval of QTgw.caas-5B was selected from BC$_1$F$_6$ generation of the ZM871/ZM895//ZM871 population (Fig. 1a, b). A heterozygous recombinant plant (HRL2925) from L2925 was self-pollinated, generating 12 heterozygous recombinant plants (designated RL1 to RL12) and 119 homozygous plants with 57 having 5B+ alleles (ZM895 genotype) and 62 having 5B− (ZM871 genotype), in which two groups of homozygous plants were used for a preliminary evaluation of the phenotypic effects of QTgw.caas-5B on TGW, GL and GW (Fig. 1c, d). Genetic backgrounds of HRL2925 were evaluated by genotyping two 5B+ and two 5B− homozygous lines generated from two kinds of homozygous plants, respectively, using the wheat 50 K SNP array developed in collaboration with the Capital-Bio, Beijing, China (https://www.capitalbiotech.com/). Twenty 5B+ and 20 5B− homozygous lines were used to measure TGW, TL and GW at different growth stages and for RNA-sequencing. In addition, 52–77 NILs from each of RL1 to RL12 were identified using QTgw.caas-5B-flanking markers to narrow the region of candidate genes (Fig. 1d; Table 2). A diverse panel of 166 cultivars (Li et al. 2019a) was used to validate the effects of QTgw.caas-5B.

---

**Springer**
**Grain sampling**

Twenty 5B+ and 20 5B− homozygous lines grown at Xinxiang in 2018–2019 were used for the study. Main stems at anthesis were marked with red tags at 09:00–11:00 am every day. Ten grains from outer florets of five spikelets in the middle regions of tagged spikes were sampled at 09:00–11:00 am at 4, 8, 12, 16, 20, 25 and 30 days post-anthesis (DPA). At each time point 10 spikes per plot were sampled, including 200 grains (20 spikes × 10 grains each spike) for each group. GL and GW were measured using image analysis software (Image-Pro Plus 6.0, [http://www.mediacy.com/](http://www.mediacy.com/)) after scanning the grain samples placed on a scanner panel with grain creases placed downwards. Following measurement, the grains were dried in an oven at 135 °C for 15 min and then at 65 °C until a constant weight. The TGW of the dried grain samples harvested at various DPA was determined, three biological replications were performed for each time point.

**RNA and DNA extraction and RNA-sequencing**

Two grains sampled from the outer florets of spikelets in the middle of tagged spikes of main stems at 4, 8, 12, 16, 20
and 25 DPA were snap-frozen in liquid nitrogen and stored at −80 °C. For 20 homozygous lines with 5B+ and 20 with 5B− selected from 119 self-pollinated homozygous plants of HRL2925, 40 grains were sampled (20 spikes × 2 grains each spike) with three biological replications at each time point.

Total RNA was isolated using the TRIzol protocol (Invitrogen, Carlsbad, CA, USA). After quality testing, a single RNA library for each sample was constructed, and the library preparations were sequenced on an Illumina HiSeq platform with 250–300 bp paired-end reads at Novogene Bioinformatics Technology in Beijing (http://www.novogene.com/). FeatureCounts v1.5.0-p3 was used to count the number of reads mapped to each gene. Then FPKM of each gene was calculated based on the length of the gene and reads count mapped. Differential expression analysis of 5B+ and 5B− genotypes were performed with three biological replications at each stage using the DESeq2 R package (1.10.1), which provides the statistical routines for determining differential expression in digital gene expression data using a model based on a negative binomial distribution. The resulting P-value was adjusted using the Benjamini and Hochberg’s approach for controlling false discovery rate. Genes with an adjusted P < 0.05 determined by DESeq2 were considered as differentially expressed. Genomic DNA was extracted from young leaves of experimental lines using the CTAB method.

Whole-genome resequencing

Quanified DNA samples of ZM871 and ZM895 were randomly fragmented by Covaris, and the fragments were collected by magnetic beads. Ligation products with end-repair and addition of 3′ adenine DNA fragments were cycled and amplified by linear isothermal rolling-circle replication and DNA nanoball technology. Sequencing of these DNA libraries was performed by the BGISEQ sequencing platform in Shenzhen Huada Gene Technology (Shenzhen, https://www.genomics.cn/). The remaining high-quality paired-end reads following filtering a high proportion of adaptors and low-quality reads in the raw data were mapped to the Chinese Spring reference genome (IWGSC 2018, https://urgi.versailles.inra.fr/blast_iwgsc/) using the Burrows-Wheeler Aligner Tool (http://bio-bwa.sourceforge.net/) with the command “mem -t 4 -k 32 -M”. SNPs and small Insertion/Deletions (InDels) were detected by GATK (https://www.broad institute.org/gatk/) with filtration parameters of “QD < 2.0 || FS > 60.0 || MQ < 40.0 || MQRankSum < −12.5 || ReadPosRankSum < −8.0” for SNP calling and “QD < 2.0 || FS > 200.0 || SOR > 10.0 || MQRankSum < −12.5 || ReadPosRankSum < −8.0” for InDel calling in the same way as the whole-genome resequencing.

Array-based SNP markers or SNPs from RNA-sequencing and whole-genome resequencing upstream of the physical location of the Kasp_5B2 locus and between Kasp_5B2 and Kasp_5B6 were converted to KASP markers for fine mapping of QTgw.caas-5B. Allele-specific and common reverse primers for each KASP marker were designed using Polymarker (Ramirez-Gonzalez et al. 2015, http://www.polymarker.info/).

Statistical analysis

For the statistical analyses in progeny tests, grain development and diversity panel, phenotypic differences between the allelic pairs were determined by Student’s t tests with SAS 9.2 software (SAS Institute Inc, Cary, NC, USA). BLUE (best linear unbiased estimation) value of the phenotypic data for each line/cultivar in each environment was used for the analyses.

Results

Generation of fine mapping population using residual heterozygous lines

A heterozygous line L2925 screened from a BC1F6 population of the ZM871/ZM895//ZM871 had homozygous background across the mapping interval spanning QTgw.caas-5B (Fig. 1a, b; Yang et al. 2020). One recombinant plant (HRL2925) from L2925 was self-pollinated and generated 12 heterozygous recombinant plants (Fig. 1c, d). The heterozygous interval was flanked by SNP markers Kasp_5B2 and Kasp_5B6 location of the marker. Genetic similarity between homozygous progenies for 5B+ and 5B− was more than 99% according to 50 K SNP array data, indicating that the segregating progenies from the HRL2925 were suitable for fine mapping (Table S2). Within each family of selfed progenies from 12 recombinant plants, homozygous non-recombinant plants, namely 5B+ NILs and 5B− NILs, were genotyped with markers according to heterozygous interval and phenotypes evaluated for fine mapping.

Phenotypic validation of NILs for QTgw.caas-5B

After a progeny test, a significant difference in TGW was detected between the genotypes 5B+ with 57 plants and 5B− with 62 plants from selfing progenies of HRL2925 (Fig. 2). In order to improve the accuracy of phenotypic evaluation, 20 homozygous lines with 5B+ and 20 with 5B− generated from two kinds of homozygous plants were evaluated at Xinxiang and Anyang (Henan province) to verify the effects of QTgw.caas-5B. Student’s t tests indicated significantly (P < 0.05) higher TGW and GL in 5B+
lines than their contrasting 5B− lines (Fig. 3). These demonstrated that the ZM895 allele at \( QTgw.caas-5B \) had a positive effect on TGW.

To further analyze the genetic effect of \( QTgw.caas-5B \), the dynamic change of grain weight and size at different developmental stages between the above 5B+ and 5B− homozygous lines were investigated. Twenty lines with 5B+ and 20 with 5B− were used to determine the differences on grain morphometric parameters. Student’s \( t \) tests indicated significantly (\( P < 0.05 \)) higher GL in 5B+ lines than those in 5B− from the 12 DPA, while 5B+ lines also had significantly (\( P < 0.05 \)) higher TGW than those of 5B− from the 25 DPA (Fig. 4, Table 1). No significant differences were observed in GW between the 5B+ and 5B− genotypes at all the developmental stages. This suggests that the increased TGW is attributed to the increased grain length in the 5B+ genotypes.

**Fine mapping of QTgw.caas-5B**

For fine mapping of \( QTgw.caas-5B \), eight new markers between \( Kasp_5B2 \) and \( Kasp_5B6 \) were developed from 660 K, 50 K SNP arrays and resequencing data (Fig. 1d, Table S1). Twelve heterozygous recombinant plants (RL1–RL12) identified from HRL2925 using 13 markers (HRL2925) grown at Xinxiang 2017–2018 (E1). ***and ns, significant at \( P < 0.001 \) and non-significant, respectively

(Fig. 1d) were analyzed for fine mapping of \( QTgw.caas-5B \). After progeny tests, significant differences in TGW were detected between 5B+ and 5B− genotypes within RL1, RL6, RL7 and RL12 (\( P < 0.05 \)), whereas there were no significant differences within the other NILs from RL2 to RL5 and RL8 to RL11 (Fig. 1d, Table 2). \( QTgw.caas-5B \) was delimited to an interval of approximately 2.0 Mb flanked by markers \( Kasp_5B29 \) and \( Kasp_5B31 \) (49.6–51.6 Mb), with 17 high-confidence genes based on gene annotations for the Chinese Spring reference genome (IWGSC 2018, https://urgi.versailles.inra.fr/blast_iwgsc/).

**Prediction of candidate genes for QTgw.caas-5B**

Based on resequencing data for the parents, SNPs or InDels were found in the coding or intron regions of \( TraesCS5B02G044500 \), \( TraesCS5B02G044600 \), \( TraesCS5B02G044700 \), \( TraesCS5B02G044900 \), \( TraesCS5B02G045500 \), \( TraesCS5B02G045800 \), \( TraesCS5B02G045900 \) and \( TraesCS5B02G046000 \), whereas the other nine high-confidence genes lacked sequence polymorphisms between two parents. SNPs are synonymous mutation in \( TraesCS5B02G044500 \), \( TraesCS5B02G044600 \), \( TraesCS5B02G044700 \), \( TraesCS5B02G045900 \) and \( TraesCS5B02G046000 \). They are not likely to cause delirious
Fig. 3 Phenotypic differences in a thousand grain weight (TGW), b grain length (GL) and c grain width (GW) between 20 homozygous lines with the 5B+ genotype and 20 with 5B−. E2 and E3, Anyang 2018–2019 and Xinxiang 2018–2019, respectively. **, *** and ns, significant at $P < 0.01$, $P < 0.001$ and non-significant, respectively.

Fig. 4 Mean thousand grain weight (TGW), grain length (GL) and grain width (GW) of 20 homozygous lines with the 5B+ genotype and 20 with 5B− at different grain development stages. * and ns, significant at $P < 0.05$ and non-significant, respectively.
effects on the proteins. Whereas, SNPs are missense mutation in **TraesCS5B02G044900**, **TraesCS5B02G045500** and **TraesCS5B02G045800**.

The results of RNA-seq indicated that only **TraesCS5B02G044800** among the 17 high-confidence genes in the 49.6–51.6 Mb region on chromosome 5B showed higher expression level in the 5B− genotype, whereas the transcript was not detected in the genotype 5B+ (Fig. 5, Table 3). The other 16 high-confidence genes, including **TraesCS5B02G044900**, **TraesCS5B02G045500** and **TraesCS5B02G045800** which had missense mutations, showed no differential expression levels between homozygous 5B+ and 5B− genotypes (Table S3). A SNP was located at 824 bp upstream of the initiation codon ATG in the promoter region of **TraesCS5B02G044800** by resequencing the parents. Thus, **TraesCS5B02G044800** was considered a candidate gene for **QTgw.caas-5B**.

### Discussion

#### Residual heterozygous recombinant lines are useful stocks for fine mapping

Many QTL for grain-related traits have been identified in different genetic backgrounds (Huang et al. 2003; Quarrie et al. 2005; Prashant et al. 2012; Cui et al. 2014; Wu et al. 2015; Ma et al. 2018; Su et al. 2018; Zhai et al. 2018; Xu et al. 2019). Most were located in large chromosome intervals due to limited numbers of markers or lack of recombination events within the targeted QTL regions. Following release of the Chinese Spring reference genome sequence (IWGSC 2018, [https://urgi.versailles.inra.fr/blast_iwgsc/](https://urgi.versailles.inra.fr/blast_iwgsc/)) and development of new sequencing technologies densely populated genetic maps can easily be constructed, and genetic information for a specific map interval can be searched. Consequently, many researchers have employed fine mapping approaches to validate QTL or narrow genomic intervals within targeted QTL regions. Brinton et al. (2017); Guan et al. (2019); Chen et al. (2020). In this study, progenies of 12 heterozygous recombinant plants were genotyped and phenotyped to fine map **QTgw.caas-5B** to an approximately 2.0 Mb physical interval containing 17 high-confidence annotated genes.

#### Validation of KASP markers flanking **QTgw.caas-5B** in a germplasm panel

The **Kasp_5B_Tgw** based on the SNP with ‘C’ in ZM895 and ‘A’ in ZM871 in the promoter region of **TraesCS5B02G044800** for **QTgw.caas-5B** was used to genotype the diversity panel of 166 cultivars, among which 48 cultivars had the ZM895 genotype, and 118 had the ZM871 genotype (Tables 4 and S3). The ZM895 genotype showed significantly (*P* < 0.05) higher TGW and GL than the ZM871 genotype in all six environments as well as BLUE value. Differences in TGW and GL between the two genotypes ranged from 2.1 to 2.9 g (5.5 to 5.9%) and 0.30 to 0.40 mm (2.0 to 3.0%), respectively (Table 4). Other three KASP markers based on SNPs in **TraesCS5B02G044900**, **TraesCS5B02G045500** and **TraesCS5B02G045800** were run on the same diversity panel, but no significant differences in TGW were observed between two genotypes. These results provided further evidence for a significant effect of **QTgw.caas-5B** on TGW, and **TraesCS5B02G044800** is probably a candidate gene for **QTgw.caas-5B**.
stages. The ZM895 genotype had significantly higher TGW and GL than ZM871 genotype in the germplasm panel assessed with the *Kasp_5B_Tgw* marker based on a SNP developed from *TraesCS5B02G044800*, thus indicating that *TraesCS5B02G044800* is a potential candidate gene for QTgw.caas-5B. According to the Chinese Spring reference genome sequence (IWGSC 2018, https://urgi.versailles.inra.fr/blast_iwgsc/), *TraesCS5B02G044800* were predicted to encode a TIR-NBS-LRR disease resistance protein and another unknown functional protein. There was no significant difference in the disease resistance between 5B+ and 5B− lines based on the data for powdery mildew and leaf rust reactions. Now, it is necessary to undertake gene over-expression and knockout studies to confirm the role of this candidate gene on TGW and GL.

### GL contributes to TGW at QTgw.caas-5B locus

Various studies suggest that the early stage of grain length development is important in determining final grain weight in wheat (Hasan et al. 2011; Guo et al. 2015; Simmonds et al. 2016; Brinton et al. 2017, 2018). This study initially detected a clear difference in GL between 5B+ and 5B− genotypes at 12 DPA, whereas a corresponding difference in TGW between 5B+ and 5B− genotypes was first observed at 25 DPA. This supported the contention that GL is a main factor contributing to grain weight (Brinton et al. 2017).  

### Applications in wheat breeding

Major stable QTL for yield-related traits with tightly linked markers is very important for molecular breeding. In this study, a QTL for TGW on chromosome 5B showed stable effects on TGW and GL across environments. Its presence in 48 accessions among a panel of 166 indicated that it had been a selected target for grain weight (or even yield) in past breeding programs, and thus *Kasp_5B_Tgw* represents a future target for marker-assisted selection to enhance grain size and weight. Moreover, the current results provide a basis for map-based cloning of the gene underlying the QTL.

### Table 2

| Line | Genotype | No. | TGW (g) | GL (mm) | GW (mm) |
|------|----------|-----|---------|---------|---------|
| RL1  | 5B+ (ZM895) | 25  | 49.7 ± 1.2b | 6.88 ± 0.05 | 3.29 ± 0.05 |
|      | 5B− (ZM871) | 27  | 47.0 ± 1.4  | 6.78 ± 0.07 | 3.25 ± 0.06 |
|      |           |     | 2.7*** | 0.10*** | 0.04* |
| RL2  | 5B+ (ZM895) | 33  | 48.7 ± 1.4  | 6.84 ± 0.05 | 3.40 ± 0.05 |
|      | 5B− (ZM871) | 42  | 48.0 ± 1.4  | 6.77 ± 0.05 | 3.38 ± 0.05 |
|      |           |     | 0.7      | 0.07*** | 0.02 |
| RL3  | 5B+ (ZM895) | 24  | 48.5 ± 1.0  | 6.84 ± 0.05 | 3.38 ± 0.03 |
|      | 5B− (ZM871) | 32  | 48.2 ± 2.1  | 6.78 ± 0.07 | 3.39 ± 0.06 |
|      |           |     | 0.3      | 0.06**  | − 0.01 |
| RL4  | 5B+ (ZM895) | 28  | 47.9 ± 2.7  | 6.89 ± 0.10 | 3.37 ± 0.09 |
|      | 5B− (ZM871) | 27  | 48.3 ± 1.7  | 6.84 ± 0.06 | 3.40 ± 0.05 |
|      |           |     | − 0.4    | 0.05*   | − 0.03 |
| RL5  | 5B+ (ZM895) | 30  | 48.8 ± 1.7  | 6.82 ± 0.11 | 3.42 ± 0.07 |
|      | 5B− (ZM871) | 29  | 48.5 ± 1.1  | 6.80 ± 0.15 | 3.40 ± 0.03 |
|      |           |     | 0.03     | 0.02     | 0.02 |
| RL6  | 5B+ (ZM895) | 43  | 48.9 ± 2.6  | 6.87 ± 0.10 | 3.39 ± 0.08 |
|      | 5B− (ZM871) | 33  | 47.0 ± 1.2  | 6.80 ± 0.09 | 3.36 ± 0.04 |
|      |           |     | 1.9**    | 0.07***  | 0.03 |
| RL7  | 5B+ (ZM895) | 29  | 49.7 ± 0.9  | 6.98 ± 0.06 | 3.44 ± 0.02 |
|      | 5B− (ZM871) | 33  | 47.5 ± 1.1  | 6.88 ± 0.07 | 3.40 ± 0.03 |
|      |           |     | 2.2**    | 0.10***  | 0.04* |
| RL8  | 5B+ (ZM895) | 33  | 48.6 ± 2.1  | 6.96 ± 0.09 | 3.40 ± 0.07 |
|      | 5B− (ZM871) | 31  | 48.5 ± 2.0  | 6.89 ± 0.10 | 3.41 ± 0.06 |
|      |           |     | 0.1      | 0.07*    | − 0.01 |
| RL9  | 5B+ (ZM895) | 29  | 48.9 ± 1.4  | 6.90 ± 0.08 | 3.41 ± 0.05 |
|      | 5B− (ZM871) | 26  | 48.4 ± 1.4  | 6.84 ± 0.09 | 3.41 ± 0.05 |
|      |           |     | 0.5      | 0.06**   | 0 |
| RL10 | 5B+ (ZM895) | 31  | 48.7 ± 1.7  | 6.95 ± 0.08 | 3.41 ± 0.04 |
|      | 5B− (ZM871) | 29  | 48.0 ± 1.1  | 6.86 ± 0.09 | 3.39 ± 0.04 |
|      |           |     | 0.7      | 0.09***  | 0.02 |
| RL11 | 5B+ (ZM895) | 24  | 48.1 ± 2.1  | 6.93 ± 0.09 | 3.39 ± 0.07 |
|      | 5B− (ZM871) | 31  | 47.8 ± 1.4  | 6.86 ± 0.07 | 3.39 ± 0.05 |
|      |           |     | 0.3      | 0.07**   | 0 |
| RL12 | 5B+ (ZM895) | 30  | 49.4 ± 1.1  | 6.88 ± 0.02 | 3.41 ± 0.04 |
|      | 5B− (ZM871) | 33  | 47.1 ± 1.2  | 6.80 ± 0.07 | 3.38 ± 0.04 |
|      |           |     | 2.3**    | 0.08**   | 0.03 |

**aNumber of lines within the corresponding genotypic group**  
**bData are means ± SD**  
**cPhenotypic difference between the means of genotypes 5B+ and 5B−. Asterisks indicate significant differences determined by Student’s *t* tests. *, **and ***, significant at *P* < 0.05, *P* < 0.01 and *P* < 0.001, respectively**

---

---

---
The online version contains supplementary material available at https://doi.org/10.1007/s00122-021-03925-9.

Acknowledgements The authors are grateful to Prof. R. A. McIntosh, Plant Breeding Institute, University of Sydney, for critical review of this manuscript. This work was funded by the CAAS Agricultural Science and Technology Innovation Program (CAAS-ZDRW202002) and the Core Research Budget of the Non-profit Governmental Research Institutions (S2021ZD04).

Author Contribution statement DZ and LY performed the experiment and wrote the paper. DZ, LY, DL, JZ and JY participated in the field trials and trait evaluation. SC, XX and XS provided extensive revision of the manuscript. ZH and YZ designed the experiment and wrote the paper. All authors read the final version of the manuscript and approved its publication.

Declaration

Conflicts of interest All authors declare that they have no conflicts of interest.

Table 3 Relative expression of TraesCS5B02G044800 in genotypes 5B+ and 5B− at different grain development stages

| Genotype     | 4 DPAa | 8 DPA   | 12 DPA  | 16 DPA  | 20 DPA  | 25 DPA  |
|--------------|--------|---------|---------|---------|---------|---------|
| 5B+ (ZM895)  | 0B     | 0B      | 0B      | 0B      | 0B      | 0B      |
| 5B− (ZM871)  | 1.02 ± 0.10Bc | 1.11 ± 0.06A | 1.18 ± 0.23A | 0.56 ± 0.18A | 0.81 ± 0.14A | 0.82 ± 0.34A |

 aDPA days post-anthesis
 bData are means ± SD
 cDifferences of expression levels between two genotypes followed by different letters are significant at P < 0.001

Table 4 Mean thousand grain weight (TGW), grain length (GL) and grain width (GW) of genotypes 5B+ and 5B− in the germplasm panel of 166 wheat cultivars grown in six environments

| Trait     | Genotypea | No. b  | E4c   | E5   | E6   | E7   | E8   | E9   | BLUEd |
|-----------|-----------|--------|-------|------|------|------|------|------|-------|
| TGW (g)   | 5B+ (ZM895) | 48     | 43.0 ± 5.4c | 43.9 ± 5.0 | 51.9 ± 5.3 | 47.7 ± 6.2 | 41.6 ± 5.0 | 41.3 ± 5.0 | 45.1 ± 4.8 |
|           | 5B− (ZM871) | 118    | 40.4 ± 4.9  | 41.5 ± 5.7  | 49.0 ± 5.4  | 45.1 ± 5.8  | 38.5 ± 4.2  | 39.0 ± 4.9  | 42.4 ± 4.8  |
|           |           | 2.6**   | 2.4*   | 2.9** | 2.6*  | 3.1*** | 2.3** | 2.7** |
| GL (cm)f  | 5B+ (ZM895) | 48     | 13.80 ± 0.74 | 13.57 ± 0.74 | 15.03 ± 0.74 | 15.02 ± 0.71 | 14.39 ± 0.68 | 14.28 ± 0.71 | 14.49 ± 0.69 |
|           | 5B− (ZM871) | 118    | 13.40 ± 0.67 | 13.17 ± 0.63 | 14.73 ± 0.68 | 14.71 ± 0.69 | 14.05 ± 0.67 | 13.93 ± 0.71 | 14.15 ± 0.64 |
|           |           | 0.40**  | 0.40*** | 0.30*  | 0.31*  | 0.34** | 0.35** | 0.34** |
| GW (cm)g  | 5B+ (ZM895) | 48     | 6.66 ± 0.41 | 6.59 ± 0.32 | 7.39 ± 0.31 | 7.22 ± 0.36 | 6.92 ± 0.35 | 6.80 ± 0.35 | 7.10 ± 0.31 |
|           | 5B− (ZM871) | 118    | 6.59 ± 0.34 | 6.49 ± 0.43 | 7.30 ± 0.36 | 7.14 ± 0.40 | 6.77 ± 0.34 | 6.63 ± 0.39 | 7.00 ± 0.34 |
|           |           | 0.07    | 0.10    | 0.09   | 0.08   | 0.15** | 0.17*  | 0.10  |

 aGenotypes identified using marker Kasp_5BTgw
 bNumber of cultivars with corresponding genotype
 cE4–E9, Anyang 2012–2013, Suixi 2012–2013, Anyang 2013–2014, Suixi 2013–2014, Suixi 2014–2015 and Shijiazhuang 2014–2015, respectively
 dBLUE Best linear unbiased estimation
 eData are shown as means ± SD
 fPhenotypic differences between two genotype 5B+ and 5B−. Asterisks indicate significant differences determined by Student’s t tests. *, ** and *** , significant at P < 0.05, P < 0.01 and P < 0.001, respectively
 gGL and GW are mean lengths and widths of 20 grains

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00122-021-03925-9.

Ethical approval We declare that these experiments complied with the ethical standards in China.

References

Brinton J, Simmonds J, Minter F, Leverington-Waite M, Snape J, Uauy C (2017) Increased pericarp cell length underlies a major quantitative trait locus for grain weight in hexaploid wheat. New Phytol 215:1026–1038
Brinton J, Simmonds J, Uauy C (2018) Ubiquitin-related genes are differentially expressed in isogenic lines contrasting for pericarp cell size and grain weight in hexaploid wheat. BMC Plant Biol 18:22
Cabral AL, Jordan MC, Larson G, Somers DJ, Humphreys DG, McCartney CA (2018) Relationship between QTL for grain shape, grain weight, test weight, milling yield, and plant height in the spring wheat cross RL4452/'AC domain'. PLoS One 13:e0190681
Cao S, Xu D, Hanif M, Xia X, He Z (2020) Genetic architecture underpinning yield component traits in wheat. Theor Appl Genet 133:1811–1823

 Springer
Chen Z, Cheng X, Chai L, Wang Z, Bian R, Li J, Zhao A, Xin M, Guo W, Hu Z, Peng H, Yao Y, Sun Q, Ni Z (2020) Dissection of genetic factors underlying grain size and fine mapping of QTgw.ca u-7D in common wheat (Triticum aestivum L.). Theor Appl Genet 133:149–162.

Cui F, Zhao C, Ding A, Li J, Wang L, Li X, Bao Y, Li J, Wang H (2014) Construction of an integrative linkage map and QTL mapping of grain yield-related traits using three related wheat RIL populations. Theor Appl Genet 127:659–675.

Dong L, Wang F, Liu T, Dong Z, Li A, Jing R, Mao L, Li Y, Liu X, Zhang K, Wang D (2014) Natural variation of TaGASR7-A1 affects grain length in common wheat under multiple cultivation conditions. Mol Breed 34:937–947.

FAO (2017) http://www.fao.org/faostat/en/.

Fischer RA, Edmeades GO (2010) Breeding and cereal yield progress. Crop Sci 50:85–98.

Gao F, Ma D, Yin G, Rasheed A, Dong Y, Xiao Y, Xia X, Wu X, He Z (2017) Genetic progress in grain yield and physiological traits in Chinese wheat cultivars of southern yellow and Huai valley since 1950. Crop Sci 57:760–773.

Guan P, Lu L, Jia L, Kabir MR, Zhang J, Lan T, Zhao Y, Xin M, Hu Z, Yao Y, Ni Z, Sun Q, Peng H (2018) Global QTL analysis identifies genomic regions on chromosomes 4A and 4B harboring stable loci for yield-related traits across different environments in wheat (Triticum aestivum L.). Front Plant Sci 9:529.

Guan P, Di N, Mu Q, Shen X, Wang Y, Wang Y, Xu K, Song W, Chen Y, Xin M, Hu Z, Guo W, Yao Y, Ni Z, Sun Q, Peng H (2019) Use of near-isogenic lines to precisely map and validate a major QTL for grain weight on chromosome 4A1 in bread wheat (Triticum aestivum L.). Theor Appl Genet 132:2367–2379.

Guo Z, Chen D, Schnurbusch T (2015) Variance components, heterobility and correlation analysis of anther and ovary size during the floral development of bread wheat. J Exp Bot 66:3099–3111.

Hanif M, Gao F, Liu J, Wen W, Zhang Y, Rasheed A, Xia X, He Z, Cao S (2016) TaTGW6-A1, an ortholog of rice TGW6, is associated with grain weight and yield in bread wheat. Mol Breed 36:1.

Hasan AK, Herrera J, Lizana C, Calderini DF (2011) Carpel weight, grain length and stabilized grain water content are physiological drivers of grain weight determination of wheat. Field Crops Res 123:241–247.

Hu M-J, Zhang H-P, Cao J-J, Zhai X-F, Wang S-X, Jiang H, Wu SY, Lu J, Chang C, Sun G-L, Ma C-X (2016) Characterization of an IAA-glucose hydrolase gene TaTGW6 associated with grain weight in common wheat (Triticum aestivum L.). Mol Breed 36:23.

Huang QX, Coster H, Galan MW, Röder MS (2003) Advanced backcross QTL analysis for the identification of quantitative trait loci alleles from wild relatives of wheat (Triticum aestivum L.). Theor Appl Genet 106:1379–1389.

International Wheat Genome Sequencing Consortium (IWGSC) (2018) Shifting the limits in wheat research and breeding using a fully annotated reference genome. Science 361:eaar7191.

Jiang Y, Jiang Q, Hao C, Hou J, Wang L, Zhang H, Zhang S, Chen X, Zhang X (2015) A yield-associated gene TaCWI, in wheat: its function, selection and evolution in global breeding revealed by haplotype analysis. Theor Appl Genet 128:131–143.

Kuchel H, Williams KJ, Langridge P, Eagles HA, Jefferies SP (2007) Genetic dissection of grain yield in bread wheat I QTL Analysis. Theor Appl Genet 115:1029–1041.

Langridge P (2013) Wheat genomics and the ambitious targets for future wheat production. Genome 56:545–547.

Li N, Li Y (2016) Signaling pathways of seed size control in plants. Curr Opin Plant Biol 33:23–32.

Li F, Wen W, Liu J, Zhang Y, Cao S, Hu Z, Rasheed A, Jin H, Zhang C, Yan J, Zhang P, Wan Y, Xian X (2019a) Genetic architecture of grain yield in bread wheat based on genome-wide association studies. BMC Plant Biol 19:168.

Li N, Xu R, Li Y (2019b) Molecular networks of seed size control in plants. Annu Rev Plant Biol 70:435–463.

Ma L, Li T, Hao C, Wang Y, Chen X, Zhang X (2016) TaGS5-3A, a grain size gene selected during wheat improvement for larger kernel and yield. Plant Biotechnol J 14:1269–1280.

Ma F, Xu Y, Ma Z, Li L, An D (2018) Genome-wide association and validation of key loci for yield-related traits in wheat founder parent Xiaoyan 6. Mol Breed 38:91.

Prashant R, Kadoo N, Desale C, Kore P, Dhaliwal HS, Chhuneja P, Gupta V (2012) Kernel morphometric traits in hexaploid wheat (Triticum aestivum L.) are modulated by intrinsic QTL × QTL and genotype × environment interactions. J Cereal Sci 56:432–439.

Quarrie SA, Steed A, Calestani C, Semikhodskii A, Lebreton C, Chinoy C, Steele N, Pjevljakusic D, Waterman E, Weyen J, Schondelmaier J, Habash DZ, Farmer P, Saker L, Clarkson DT, Abugaliyeva A, Yessimbekova M, Turuspekov Y, Abuagaliyeva S, Tuberosa R, Sanguineti MC, Hollington PA, Aragues R, Royo A, Dodig D (2005) A high-density genetic map of hexaploid wheat (Triticum aestivum L.) from the cross Chinese spring × SQ1 and its use to compare QTLs for grain yield across a range of environments. Theor Appl Genet 110:865–880.

Ramirez-Gonzalez RH, Uauy C, Caccamo M (2015) PolyMarker: a fast polyploidy primer design pipeline. Bioinformatics 31:2038–2039.

Sajjad M, Ma X, Habibullah Khan S, Shoaib M, Song Y, Yang W, Zhang A, Liu D (2017) TaFlo2-A1, an ortholog of rice Flo2, is associated with thousand grain weight in bread wheat (Triticum aestivum L.). BMC Plant Biol 17:164.

Simmonds J, Scott P, Leverington-Waite M, Turner AS, Brinton J, Korzun V, Snape J, Uauy C (2014) Identification and independent validation of a stable yield and thousand grain weight QTL on chromosome 6A of hexaploid wheat (Triticum aestivum L.). BMC Plant Biol 14:191.

Simmonds J, Scott P, Brinton J, Mestre TC, Bush M, Del Blanco A, Dubcovsky J, Uauy C (2016) A splice acceptor site mutation in TaGW2-A1 increases thousand grain weight in bread wheat (Triticum aestivum L.). BMC Genet 17:164.

Sun C, Zhang F, Zhang X, Dong Z, Cui D, Chen F (2017) Genome-wide association study for 13 agronomic traits reveals distribution of superior alleles in bread wheat from the yellow and Huai valley of China. Plant Biotechnol J 15:953–969.

Wang S, Zhang X, Chen F, Cui D (2015) A single-nucleotide polymorphism of TaGS5 gene revealed its association with kernel weight in Chinese bread wheat. Front Plant Sci 6:1166.

Wang S, Yan W, Wang Y, Liu H, Cui D, Chen F (2016) Haplotypes of the TaGS5-A1 gene are associated with thousand-kernel weight in Chinese bread wheat. Front Plant Sci 7:783.

Wu Q, Chen Y, Zhou S, Fu L, Chen J, Xiao Y, Zhang D, Ouyang S, Zhao X, Cui Y, Zhang D, Liang Y, Wang Z, Xie J, Qin J, Wang G, Li D, Huang Y, Yu M, Lu P, Wang L, Wang L, Wang H, Dang C, Li J, Zhang Y, Peng H, Yuan C, You M, Sun Q, Wang J, Wang L, Luo M, Han J, Liu Z (2015) High-density genetic linkage map construction and QTL mapping of grain shape and...
size in the wheat population Yanda 1817 x Beinong6. PLoS One 10:e0118144
Xu D, Wen W, Fu L, Li F, Li J, Xie L, Xia X, Ni Z, He Z, Cao S (2019) Genetic dissection of a major QTL for kernel weight spanning the Rht-B1 locus in bread wheat. Theor Appl Genet 132:3191–3200
Yang J, Zhou YJ, Wu QH, Chen YX, Zhang PP, Zhang YE, Hu WG, Wang XC, Zhao H, Dong LL, Han J, Liu Z, Cao TJ (2019) Molecular characterization of a novel TaGL3-5A allele and its association with grain length in wheat (Triticum aestivum L.). Theor Appl Genet 132:1799–1814
Yang L, Zhao D, Meng Z, Xu K, Yan J, Xia X, Cao S, Tian Y, He Z, Zhang Y (2020) QTL mapping for grain yield-related traits in bread wheat via SNP-based selective genotyping. Theor Appl Genet 133:857–872
Yue A, Li A, Mao X, Chang X, Li R, Jing R (2015) Identification and development of a functional marker from 6-SFT-A2 associated with grain weight in wheat. Mol Breed 35:63
Zhai H, Feng Z, Du X, Song Y, Liu X, Qi Z, Song L, Li J, Li L, Peng H, Hu Z, Yao Y, Xin M, Xiao S, Sun Q, Ni Z (2018) A novel allele of TaGW2-A1 is located in a finely mapped QTL that increases grain weight but decreases grain number in wheat (Triticum aestivum L.). Theor Appl Genet 131:539–553
Zhang L, Zhao YL, Gao LF, Zhao GY, Zhou RH, Zhang BS, Jia JZ (2012) TaCKX6-D1, the ortholog of rice OsCKX2, is associated with grain weight in hexaploid wheat. New Phytol 195:574–584
Zhang Y, Liu J, Xia X, He Z (2014) TaGS-D1, an ortholog of rice OsGS3, is associated with grain weight and grain length in common wheat. Mol Breed 34:1097–1107

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.