ORIGINAL ARTICLE

Dissection of genetic factors underlying grain size and fine mapping of *QTgw.cau-7D* in common wheat (*Triticum aestivum* L.)

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Abstract

Key message **Thirty environmentally stable QTL controlling grain size and/or plant height were identified, among which *QTgw.cau-7D* was delimited into the physical interval of approximately 4.4 Mb.**

Abstract Grain size and plant height (PHT) are important agronomic traits in wheat breeding. To dissect the genetic basis of these traits, we conducted a quantitative trait locus (QTL) analysis using recombinant inbred lines (RILs). In total, 30 environmentally stable QTL for thousand grain weight (TGW), grain length (GL), grain width (GW) and PHT were detected. Notably, one major pleiotropic QTL on chromosome arm 3DS explained the highest phenotypic variance for TGW, GL and PHT, and two stable QTL (*QGw.cau-4B* and *QGw.cau-7D*) on chromosome arms 4BS and 7DS contributed greater effects for GW. Furthermore, the stable QTL controlling grain size (*QTgw.cau-7D* and *QGw.cau-7D*) were delimited into the physical interval of approximately 4.4 Mb harboring 56 annotated genes. The elite NILs of *QTgw.cau-7D* increased TGW by 12.79–21.75% and GW by 4.10–8.47% across all three environments. Collectively, these results provide further insight into the genetic basis of TGW, GL, GW and PHT, and the fine-mapped *QTgw.cau-7D* will be an attractive target for positional cloning and marker-assisted selection in wheat breeding programs.

Introduction

Wheat is one of the most important food crops worldwide, providing approximately 20% of the calories for human (http://faostat.fao.org). To ensure global food security, genetic improvement in grain yield will remain the principal aim of wheat breeding programs (Hanif et al. 2015; Kumari

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et al. 2018). Grain yield is a complex quantitative trait that can be broken down into three individual components (Simmonds et al. 2014, 2016). As one of the components of grain yield, thousand grain weight (TGW) is principally influenced by grain morphometric parameters (e.g., grain length (GL), grain width (GW), grain thickness (GT) and grain surface area) and grain filling (grain filling rate and duration) (Guan et al. 2019; Xie et al. 2015; Zanke et al. 2015; Zhai et al. 2018). These traits are inherited in a relatively stable manner and exhibit increased heritability values than overall yield (Kuchel et al. 2007). Therefore, the exploitation of genetic variation for TGW and related traits is a promising approach to improve wheat yield (Wurschum et al. 2018).

Recently, the availability of resources, including draft and complete genome sequences (International Wheat Genome Sequencing 2014, 2018), high-density single nucleotide polymorphism (SNP) arrays (Wang et al. 2014; Winfield et al. 2016) and transcriptomic databases (Borrill et al. 2016; Ramirez-Gonzalez et al. 2018), has led to the development of a powerful approach to identify QTL controlling grain size in wheat, including TGW, GL and GW (Li and Yang 2017). A large number of QTL for grain size have been identified and characterized (Cui et al.

2014; Guan et al. 2018; Su et al. 2018; Sukumaran et al. 2018; Zanke et al. 2015; Zhai et al. 2018). Moreover, some QTL have been fine-mapped via constructing advanced separated populations. For example, Brinton et al. (2017) identified a major QTL for grain weight and narrowed the genetic interval to a 4.3 cM using near-isogenic lines (NILs). However, wheat has a large and polyploid genome, which impedes map-based cloning of genes for grain size (Li and Yang 2017).

The last decade witnessed a blossoming of genetic research on grain size mainly through the use of forward genetics approaches in the model plant rice (Li and Yang 2017), and these genes are mostly involved in three pathways: proteasomal degradation, G-protein signaling and phytohormone signaling (Li and Li 2016). Isolating several genes associated with grain size in wheat by using a homology-based approach has provided insight into the molecular basis of grain size in wheat (Nadolska-Orczyk et al. 2017), such as *TaGASR7* (Dong et al. 2014), *TaCwi* (Jiang et al. 2015), *TaGW2-A1* (Simmonds et al. 2016), *TaGS5-3A* (Ma et al. 2016), *TaTGW6-A1* (Hanif et al. 2015), *TaGS-D1* (Zhang et al. 2014b), *TaCKX6-D1* (Zhang et al. 2012) and *TaGL3-5A* (Yang et al. 2019). Despite these advances, our understanding of the genetic control of grain size remains an area that requires further elucidation.

Plant height (PHT) is another important trait for selection in both crop domestication and commercial crop improvement (Peng et al. 1999; Zhang et al. 2014a). With the boosting of the "Green Revolution", major genes conferring reduced plant height were introduced to the global wheat industry (Kuchel et al. 2007), and several studies have revealed that plant height is positively correlated with TGW (Huang et al. 2003; Roder et al. 2008; Tian et al. 2017b). To date, 25 PHT genes have been reported in wheat (McIntosh et al. 2017; Mo et al. 2018). Among them, *Rht1* loci have been verified strongly linked to the major QTL for TGW, and the positive effects were associated with the wild-type alleles, *Rht-B1a* and *Rht-D1a* (Zhang et al. 2013). *Rht8*, which is located on chromosome arm 2DS, is also extensively utilized in wheat breeding globally (Korzun et al. 1998). The reduction in PHT by *Rht8* was achieved predominantly without significant negative effects on TGW (Korzun et al. 1998). Therefore, genetic loci associated with TGW and PHT that are obtained by QTL mapping can provide a clear understanding of their genetic relationships (Guan et al. 2018).

In the present study, we performed QTL analysis of TGW, GL, GW and PHT using a recombinant inbred line (RIL) population from the cross of Hesheng2 and 4332, with the purpose to understand the genetic basis underlying these traits. Furthermore, to validate the genetic effects of *QTgw.cau-7D* and precisely map this QTL, residual heterozygous line (RIL120) was used to screen recombinants and develop NILs for analysis, which will be beneficial for map-based cloning and molecular marker-assistant selection.

Materials and methods

Plant materials

The RIL population included 271 lines derived from a crossing between Hesheng2hao (HS2) and Nongda4332 (4332). The population was developed by the single seed descent method and was advanced to the F_8 generation. HS2 is a high-yield, high-quality line, obtained by in vitro culture together with an induced mutation technique. 4332 is an accession characterized by small grains but better agronomic traits.

RIL120 (F_8 generation), exhibited a residual heterozygous genotype within the candidate interval of *QTgw.cau-7D*, was self-pollinated twice (F_{10} generation) to screen recombination lines and subsequently generated NILs. NIL families were constructed by self-fertilized recombinants, which were subsequently named NP1–NP8. NILs that are homozygous for HS2 (7D+) and 4332 (7D−) were identified using markers flanking *QTgw.cau-7D* and further used for evaluating the phenotypic effects of this QTL on TGW, GL and GW. Three sets of NIL were selected from NP1, NP4 and NP8 homozygous progenies, respectively. Each NIL set comprised 5–10 7D+ lines and 5–10 7D− lines.

Field trials and traits measurement

The RIL population (F_8 and F_9) and the two parents were planted in the field at Shandong, Hebei and Shanxi over two consecutive crop seasons (2014–2015 and 2015–2016) (Supplementary Table S1). The field trials were conducted in randomized complete block design with three replications. Each line in a block consisted of a plot of two rows, each row was 1.5 m long and 20 cm apart. NIL families were sown in rows 1.5 m long and 0.3 m apart at a sowing rate of 20 seeds per row at Hebei during the 2016–2017 growing season. Three NIL pairs were evaluated at three locations (Beijing, Hebei and Shanxi) during 2017–2018 crop season and grown in two-row plots in a randomized complete block design with three replications.

For RILs, PHT was measured from 6 to 10 plants of each lines at maturity. For RILs and three NIL pairs, 10–20 main spikes of each line were sampled and threshed. Wanshen SC-G seed detector (Hangzhou Wanshen Detection Technology Co., Ltd) was used to record TGW, GL and GW. The mean values of each trait over three replications were used to analyze data for the individual environment. TGW, GL and GW of the NIL families were measured on a single plant basis.

Data analysis

Basic statistical analysis and phenotypic correlation coefficients were conducted using SPSS version 20.0 (SPSS, Chicago, IL, USA). Analysis of variance (ANOVA) was performed using R software (V. 3.2.2) for the data from all the environments for each trait. The broad-sense heritability (H_B^2) for each trait was estimated in R software (V. 3.2.2) with the lme4 package following the formula: $H_B^2 = \sigma_g^2/(\sigma_g^2 + \sigma_{ge}^2/n + \sigma^2/nr)$, where σ_g^2 is the genotypic effect, σ_{ge}^2 is the genotype by environmental effect, σ^2 is the residual error, n is the number of environments, and r is the number of replicates. The best linear unbiased prediction (BLUP) for all four traits across six environments was calculated using SAS9.2 (SAS Institute Inc., North Carolina, USA) with the PROC MIXED procedure. The Shapiro–Wilk test was performed with R software (V. 3.2.2) to test departures from normal distribution. In the progeny test analysis, the phenotypic difference between two alleles was calculated by Student's t test.

Genetic map construction

The Illumina iSelect 90 K Infinium wheat SNP genotyping array was used to genotype the RIL population and the parents. High-quality DNA was extracted according to the traditional CTAB method (hexadecyltrimethy ammonium bromide) (Allen et al. 2006). In addition, polymorphic SSRs were used to genotype the RIL population to confirm the specific chromosome. Primer sequences of most of SSR markers were obtained from GrainGenes (https://wheat.pw. usda.gov/cgi-bin/GG3/). The linkage maps were constructed by JoinMap4.0 (Van Ooijen 2006) and RECORD 2.0 (Van Os et al. 2005). Maximum likelihood mapping algorithm and Kosambi's function (Kosambi 1943) were used to determine marker order and distance, respectively. Unique SNP markers that mapped on each chromosome were positioned onto the newly released reference genome sequence of Chinese Spring by blasting their flanking sequences against the IWGSC RefSeqv1.0 (https://urgi.versailles.inra.fr/blast _iwgsc/blast.php).

QTL analysis

QTL mapping was conducted using traits data in each environment and the adjusted mean values (BLUP) in software Windows QTL Cartographer Version 2.5 (Wang et al. 2012a). Composite interval mapping (CIM) (Zeng 1994) and model 6 of the Zmapqtl procedure (Basten et al. 1997) determined position and additive effects of QTL. Walking speed, control markers and window size were 1 cM, 5 and 10 cM, respectively. Empirical threshold LOD values were calculated based on 1000 random permutation tests ($P = 0.05$) (Yan et al. 2006). QTL sharing similar confidence intervals

(± 2 LOD away from the peaks of likelihood ratios) were considered identical QTL. QTL were named according to McIntosh et al. (2017).

SSR and InDel markers development

The flanking SNP markers of the *QTgw.cau-7D* on chromosome arm 7DS were used to blast the sequence and ensure the physical locations in ATGSP (http://aegilops.wheat .ucdavis.edu/ATGSP/) and IWGSC (https://urgi.versailles .inra.fr/blast_iwgsc/blast.php). Sequences harboring SSR regions with at least 5 dinucleotide or trinucleotide repeats were detected using SSR Hunter 1.3 (Li and Wan 2005). According to the resequencing results of two parents (the concrete method refer to Chai et al. (2018), and the data has been submitted to NCBI (Submission ID: SUB5488747)), we searched sequences containing insertion/deletion in the QTL interval. Finally, these selected sequences were used to design SSR and InDel markers in Primer3 v0.4.0 (http:// bioinfo.ut.ee/primer3-0.4.0/). The PCR reaction system included 5 µl $2 \times Taq$ PCR StarMix, 2 µl primer (mixture of left and right primer, 2 µM), 2 µl DNA template (50–100 ng/µl) and 1 µl H_2O. The PCR cycling was conducted as follows: 94 °C for 5 min; 35 cycles of 94 °C denaturation for 30 s, 55–58 °C annealing for 30 s, and 72 °C extending for 30 s; and finally, 72 °C for 10 min. An 8% non-denatured polyacrylamide gel electrophoresis (PAGE) is a common method for identifying the length of the polymorphism (Marklund et al. 2009). The SSR and InDel markers used in this study were listed in the Supplementary Table S9.

Results

Phenotypic performance

Phenotypic performance of four traits (TGW, GL, GW and PHT) from two parents and RIL population across six individual environments are shown in Supplementary Table S2. Compared with 4332, HS2 had significantly higher TGW, GL, GW and PHT in all environments (Fig. 1, Supplementary Table S2). The Shapiro–Wilk test and Pearson' correlation coefficients were conducted based on the mean value collected from individual environment as well as BLUP values, and consistent results were obtained (Supplementary Fig. S1, Supplementary Table S2, Supplementary Table S3). Briefly, TGW and GW displayed approximate normal distributions in multiple environments, whereas GL and PHT exhibited an obvious bimodal pattern in all environments, suggesting that GL and PHT were controlled by major genes (Supplementary Fig. S1, Supplementary Table S2). In addition, for the combined analysis, significant positive correlations ($P < 0.05$) were observed between TGW and other three
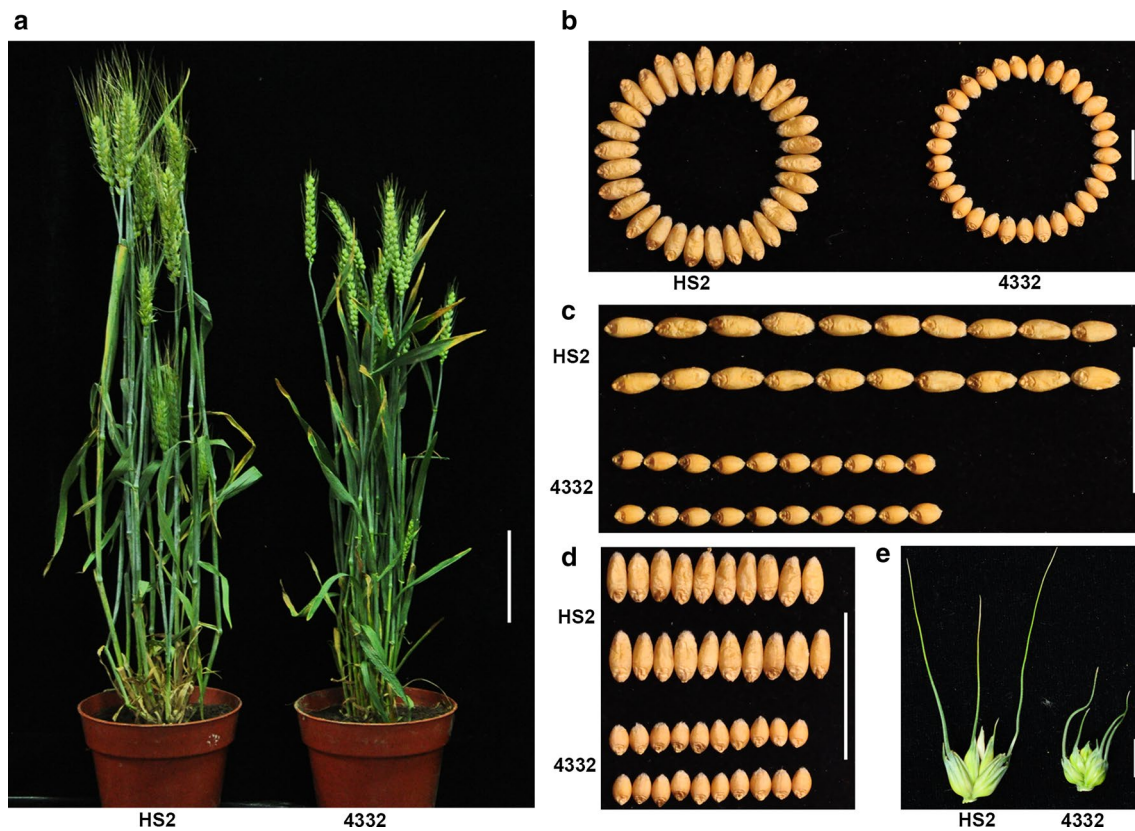
Fig. 1 Phenotypic characterization of two parents used for RIL population construction. **a** HS2 and 4332 plants at the mature stage. Bars, 10 cm. **b**, **c**, **d** Comparisons of grains between HS2 and 4332. Bars, 1 cm. **e** Spikelet of HS2 and 4332. Bars, 1 cm

traits, including GL, GW and PHT. Notably, GL had the highest positive correlation with PHT ($r = 0.899$). Whereas, the correlations between GW and PHT were relatively weak ($r = 0.151$), as well as between GW and GL ($r = 0.257$) (Supplementary Table S3). ANOVA analysis indicated significant differences ($P < 0.0001$) among RIL genotypes and among environments for the four traits (Supplementary Table S4). Broad sense heritabilities of four traits were all greater than 0.90 (Supplementary Table S4), indicating that these traits were mainly determined by genetic factors.

Genetic linkage map construction

To construct a high-density genetic linkage map, two parents and RIL population were genotyped by 90 K SNP array. A total of 9185 SNPs showed polymorphisms between HS2 and 4332. After removing SNPs with more than 20% missing data, 9002 SNPs were used for linkage analysis. In addition, forty polymorphic SSR markers were used to anchored chromosomes. The resultant linkage map comprised 27 linkage groups that consisted of 8868 SNP markers and 38 SSR markers, spanning 3200.3 cM in length, with an average interval distance of 1.3 cM between the adjacent markers (Supplementary

Fig. S2, Supplementary Table S5). Chromosomes 2D, 3D, 5D, 6D and 7D were integrated by more than one linkage group (Supplementary Fig. S2). Remarkably, the resultant genetic linkage map included six larger gaps (≥ 25 cM), which were distributed on chromosomes 1B (30 cM), 2B (29.9 cM), 2D (27.8 cM), 4D (25 cM), 5B (36.8 cM) and 7D (27.6 cM) (Supplementary Table S6). The number of mapped markers on A, B and D genomes was 3888, 4502 and 516, respectively (Supplementary Table S5). A total of 2502 unique markers in the linkage map were used for QTL analysis (Supplementary Table S6).

QTL analysis

A total of 57 QTL were identified for TGW, GL, GW and PHT. Among these QTL, 30 were stable QTL that could be detected in more than three individual environments and BLUP analysis (Fig. 2, Table 1, Supplementary Table S7) and the remaining 27 were putative QTL (Supplementary Table S8).

TGW

A total of eight stable QTL for TGW were identified, and HS2 contributed increasing alleles for all of them (Fig. 2, Table 1, Supplementary Table S7). Remarkably, the major stable QTL on chromosome arm 3DS, *QTgw.cau-3D1*, was repeatedly detected in all of the environments as well as BLUP data, explaining the largest phenotypic variance (35.93–59.57%) for TGW. *QTgw.cau-4B-1* and *QTgw.cau-4B-2* were two adjacent stable QTL that explained 13.17% and 7.82% of the variation observed for TGW, respectively, in the analysis of BLUP. The other five stable QTL detected on chromosome arms 2BL, 6BL, 6DS, 7AL and 7DS had relatively minor effects, with LOD scores ranging from 2.53 to 5.13.

GL

Ten stable QTL on eight chromosomes were detected for GL, and HS2 provided the beneficial alleles at all loci (Fig. 2, Table 1, Supplementary Table S7). The most stable QTL with major effect was identified on chromosome arm 3DS (*QGl.cau-3D1*) in all of the six environments and contributed up to 81.25–86.31% of phenotypic variance for GL. This locus was co-located with the major QTL for TGW on chromosome arm 3DS (*QTgw.cau-3D1*). *QGl.cau-5B* was also significant in all environments evaluated and explained 3.15% of the total phenotypic variance for the combined analysis.

GW

Five stable QTL associated with GW were detected on chromosome arms 2BL, 4BS, 6DS, 7AL and 7DS, separately (Fig. 2, Table 1, Supplementary Table S7). The alleles for increased GW at these five loci were provided by HS2. Chromosome arm 4BS had the most major stable QTL for GW (*QGw.cau-4B*), elucidating 4.99–42.34% of the phenotypic variance. This locus was co-located with the major stable QTL for TGW, *QTgw.cau-4B-1*. Another major stable QTL, *QGw.cau-7D*, was bound by the marker interval *Xcau.7D-10–BobWhite_rep_c65034_450* with a LOD score 5.40–12.69 and explained 5.63–18.83% of the total phenotypic variance of GW. For the combined analysis, the phenotypic variation explained by the remaining three stable QTL (*QGw.cau-2B*, *QGw.cau-6D1* and *QGw.cau-7A-2*) was 6.94, 3.56 and 4.03%, respectively.

PHT

Seven stable QTL on chromosome arms 2AL, 3AS, 3DS, 4AL, 5BL and 6DS were found for PHT (Fig. 2, Table 1, Supplementary Table S7). Consistent with the frequency distribution results, a solely major stable QTL (*QPht.cau-3D1*) contributing 78.36–87.08% of the phenotypic variation was found across all the environments. This locus was co-located with the major stable QTL for TGW and GL on chromosome arm 3DS. The other six minor stable QTL contributed 0.09–2.21% phenotypic variation, respectively. The alleles for increased PHT at five loci, *QPht.cau-3D.1*, *QPht.cau-3D.2*, *QPht.cau-4A*, *QPht.cau-5B* and *QPht.cau-6D*, were provided by HS2, whereas 4332 contributed the positive alleles for *QPht.cau-2A* and *QPht.cau-3A*.

Fine mapping of *QTgw.cau-7D*

The residual heterozygous line (RHL) provides an efficient method for fine mapping of QTL without extensive backcrossing, especially for self-pollinated crops (Liu et al. 2018; Tuinstra et al. 1997). Fortunately, one RHL (RIL120) carrying heterozygous genotypes across the mapping interval of *QTgw.cau-7D* was successfully screened from F_8 generation. Thus, *QTgw.cau-7D* was selected for fine mapping. To validate this identified QTL, new polymorphic markers between HS2 and 4332 were developed to genotype the RIL population (Supplementary Table S9). The resultant genetic linkage map consisted of 23 markers covering a genetic distance of 69.43 cM, including 14 SSR, 3 InDel and 6 SNP markers (Fig. 3b, Supplementary Table S10). In the subsequent remapping, *QTgw.cau-7D* was located to the interval flanked by markers *Xcau.7D-3* and *Xcau.7D-17* (~23 Mb), and explained 5.10–12.56% and 5.25–7.56% of the phenotypic variance for TGW and GW, respectively (Fig. 3). Considering of the larger contribution of *QTgw.cau-3D1* for TGW (35.93–59.57%), we excluded the influence of this major QTL and reanalyzed the phenotypic effect of different alleles for *QTgw.cau-7D*. Analysis of RIL lines harboring either HS2 or 4332 homozygous alleles across the wider QTL region of *QTgw.cau-7D* demonstrated that HS2 allele provided a greater TGW and GW than 4332 allele, which confirmed that the *Xcau.7D-3-Xcau.7D-17* interval contained a functional unit controlling TGW and GW (Supplementary Fig. S3).

To narrow down the interval of *QTgw.cau-7D* and independently validate its effect, two pairs of NILs with alternative haplotypes flanked by *Xcau.7D-3-Xcau.7D-17* were genotyped to identify the consistency of genetic background using the 660 K SNP array (http://wheat.pw.usda.gov/ggpages/topics/Wheat660_SNP_array_developed_by_CAAS.pdf). Greater than 97% genetic similarity between each pair of NILs indicated that the segregated progenies of RIL120 were suitable for fine mapping (Supplementary Table S11). Meanwhile, eight recombinants representing six recombination types were identified from F_{10} population by using seven markers (Fig. 4). Subsequently, these eight recombinants were planted to generate segregation families (designated as
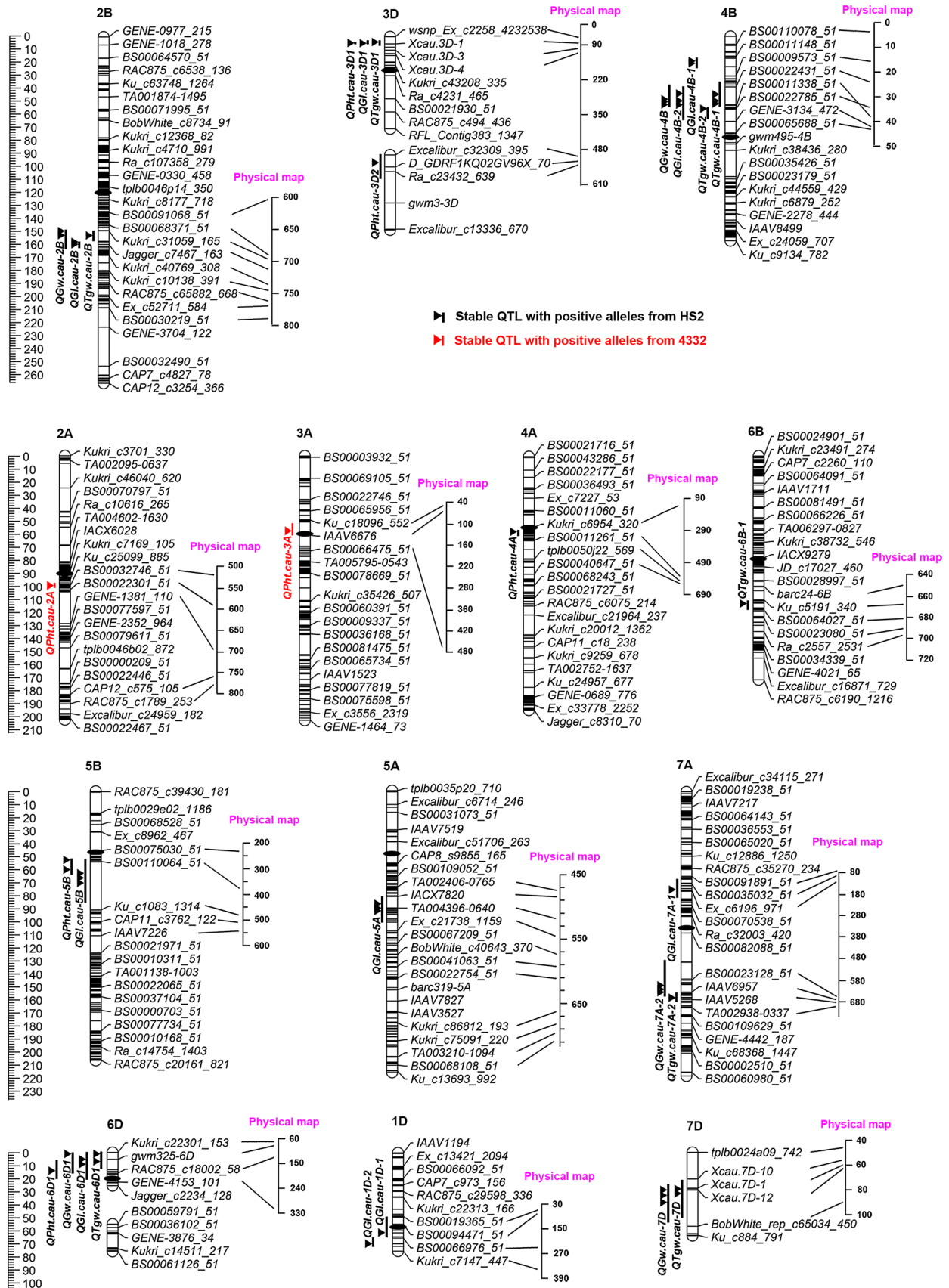
◄**Fig. 2** Genetic and physical locations of QTL regions associated with thousand grain weight (TGW), grain length (GL), grain weight (GW) and plant height (PHT). Uniform centimorgan (cM) scales are shown on the left. Physical maps are shown on the right of each genetic map. Black ellipses indicate the approximate positions of the centromeres. Vertical bars and triangles represent the confidence interval and peak position for the location of each QTL, respectively. Black and red indicate environmentally stable QTL with positive alleles from parent HS2 and parent 4332, respectively. Double-headed triangles or arrows indicate the QTL regions characterized by QTL clusters

NP1–NP8) (Fig. 4). Within each family, homozygous nonrecombinant lines, namely 7D+ NILs (with HS2 allele) and 7D− NILs (with 4332 allele), were genotyped with appropriate markers and phenotypically evaluated. After a progeny test, a significant phenotypic difference in TGW was detected between 7D+ and 7D− NILs within the populations NP1 to NP7 ($P < 0.05$), whereas nonsignificant effects for TGW were observed within family NP8 (Fig. 4). Taken together, *QTgw.cau-7D* was delimited into the interval of

Table 1 The QTL regions harboring environmentally stable QTL for thousand grain weight (TGW), grain length (GL), grain weight (GW) and plant height (PHT) in the HS2/4332 RIL population

Chromosome	Interval (cM)	Interval (Mb)[a]	Associated trait[b]	Included stable QTL[c]	Detected environment[d]	References
1D	48.9–71.9	37.2–308.5	GL (+)	*QGl.cau-1D-1; QGl.cau-1D-1*	E1/E2/E3/B	Cui et al. (2016)
2A	90.7–128.0	176.9–690.9	PHT (−)	*QPht.cau-2A*	E1/E2/E4/E5/B	
2B	138.3–173.5	610.0–747.6	TGW (+)	*QTgw.cau-2B*	E2/E3/E6/B	Cabral et al. (2018), Carter et al. (2012), Deng et al. (2017)
			GL (+)	*QGl.cau-2B*	E1/E2/E3/E5/E6/B	Zhai et al. (2016)
			GW (+)	*QGw.cau-2B*	E2/E3/E4/E5/E6/B	
3A	51.3–73.3	47.0–371.6	PHT (−)	*QPht.cau-3A*	E4/E5/E5/B	Eriksen et al. (2003)
3D1	0.0–16.0	90.8–170.5	TGW (+)	*QTgw.cau-3D1*	E1/E2/E3/E4/E5/E6/B	McCartney et al. (2005)
			GL (+)	*QGl.cau-3D1*	E1/E2/E3/E4/E5/E6/B	Wu et al. (2015)
			PHT (+)	*QPht.cau-3D1*	E1/E2/E3/E4/E5/E6/B	
3D2	0.0–36.9	531.1–559.2	PHT (+)	*QPht.cau-3D2*	E1/E2/E4/E5/E6/B	
4A	58.1–78.2	460.2–576.6	PHT (+)	*QPht.cau-4A*	E1/E2/E3/E4/E5/E6/B	
4B	38.3–65.4	245.5–432.2	TGW (+)	*QTgw.cau-4B-1; QTgw.cau-4B-2*	E1/E2/E3/E4/E5/E6/B	Chen et al. (2014), Liu et al. (2014), Kumar et al. (2016), Guan et al. (2018)
			GL (+)	*QGl.cau-4B-1; QGl.cau-4B-2*	E1/E2/E3/E4/E5/B	Kumar et al. (2016)
			GW (+)	*QGw.cau-4B*	E1/E2/E3/E4/E5/E6/B	Liu et al. (2014)
5A	78.3–101.1	479.0–549.4	GL (+)	*QGl.cau-5A*	E1/E2/E3/E4/E5/B	Wu et al. (2015)
5B	31.3–91.6	31.1–478.3	GL (+)	*QGl.cau-5B*	E1/E2/E3/E4/E5/E6/B	Kumar et al. (2016)
			PHT (+)	*QPht.cau-5B*	E1/E2/E3/E4/E6/B	Wang et al. (2010)
6B	111.2–123.6	668.8–686.7	TGW (+)	*QTgw.cau-6B-1*	E3/E4/E6/B	Ramya et al. (2010)
6D	0–18.0	61.2–292.1	TGW (+)	*QTgw.cau-6D1*	E1/E3/E4/E5/E6/B	Wang et al. (2009)
			GL (+)	*QGl.cau-6D1*	E2/E4/E5/E6/B	
			GW (+)	*QGw.cau-6D1*	E1/E3/E6/B	Xiao et al. (2011)
			PHT (+)	*QPht.cau-6D1*	E3/E4/E5/E6/B	Guan et al. (2018)
7A	67.1–83.3	83.7–116.6	GL (+)	*QGl.cau-7A-1*	E1/E2/E3/E5/B	Zhai et al. (2016)
	147.9–171.9	669.7–689.9	TGW (+)	*QTgw.cau-7A-2*	E1/E2/E3/E4/E6/B	Quarrie et al. (2006), Guan et al. (2018)
			GW (+)	*QGw.cau-7A-2*	E1/E2/E3/E4/E6/B	Kumar et al. (2016)
7D	0.0–57.0	44.5–92.6	TGW (+)	*QTgw.cau-7D*	E1/E2/E4/E6/B	Roder et al. (2008), Kumar et al. (2016)
			GW (+)	*QGw.cau-7D*	E1/E2/E4/E5/B	Kumar et al. (2016)

[a]The corresponding physical intervals (Mb) of the QTL regions were obtained by blasting the flanking sequences of SNP markers against the Chinese Spring RefSeq v1.0 sequence. [b] The traits include thousand grain weight (TGW), grain length (GL), grain weight (GW) and plant height (PHT); (+) represent the increasing allele contributed by HS2 and (−) represent the increasing allele contributed by 4332. [c] Stable QTL were detected in above three environments as well as BLUPs. [d] E1, 2014–2015 Hebei; E2, 2014–2015 Shandong; E3, 2014–2015 Shanxi; E4, 2015–2016 Hebei; E5, 2015–2016 Shandong; E6, 2015–2016 Shanxi. B indicates the combined QTL analysis based on BLUP value

Fig. 3 Remapped *QTgw.cau-7D* used saturated genetic linkage map. **a** QTL mapping for TGW in six individual environments (E1–E6) and BLUP using a saturated genetic map. **b** Saturated genetic map of chromosome 7DS in the RIL population. **c** Corresponding physical position according to the Chinese Spring IWGSC RefSeq v1.0 sequence. The interval of genetic map highlighted in blue and vertical bars colored in black on the right side represents the interval of *QTgw.cau-7D*. E1, 2014–2015 Hebei; E2, 2014–2015 Shandong; E3, 2014–2015 Shanxi; E4, 2015–2016 Hebei; E5, 2015–2016 Shandong; E6, 2015–2016 Shanxi
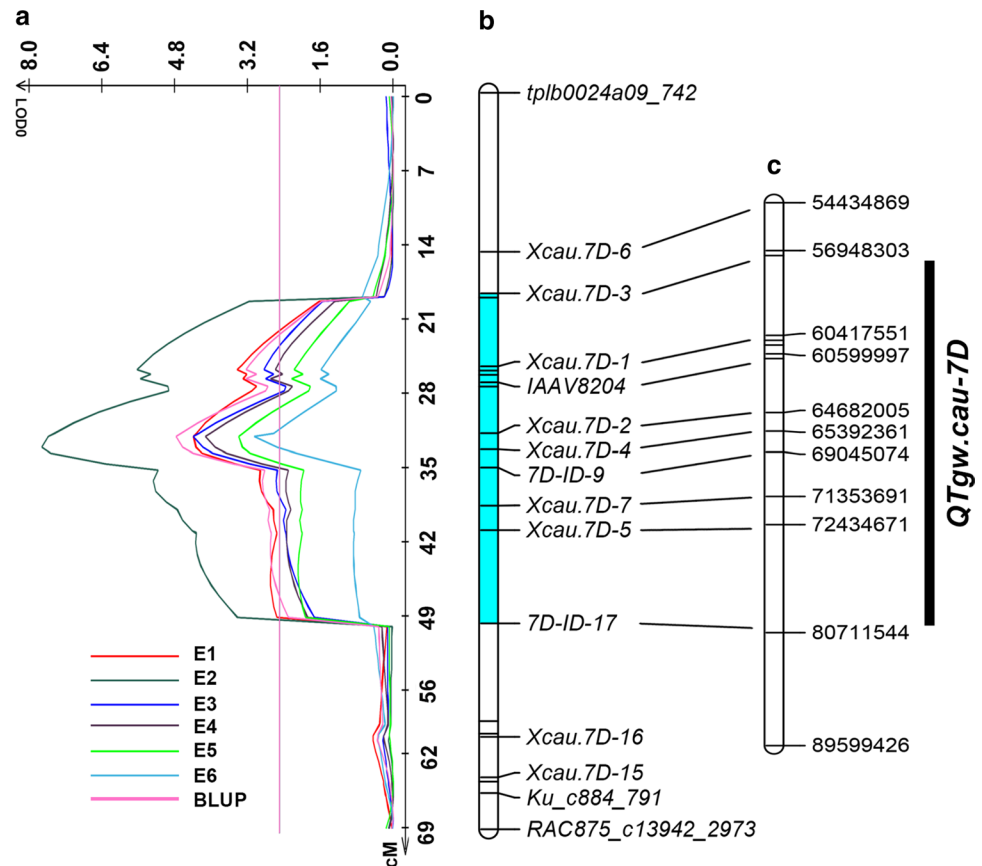
approximately 4.4 Mb flanked by the markers *Xcau.7D-2* and *7D-ID-9* (Figs. 3b, c, 4).

In the progeny test, the most informative comparisons were obtained from families NP1, NP4 and NP8 (Fig. 4). To improve the accuracy of phenotypic identification of recombinants, three sets of NIL pairs from each family (NP1, NP4 and NP8) were used to verify the effects of *QTgw.cau-7D*. Across all three environments, the average TGW of 7D+ was 12.79% to 21.75% ($P < 0.001$) higher than that of 7D− in the pairs derived from NP1 and NP4 (Table 2). As expected, the difference in GW was also observed in these two sets of NIL pairs, and 7D+ exhibited the higher value with a significant increase of 4.10–8.47% (Table 2). Interestingly, the GL of 7D+ was significantly increased compared with that of 7D− except one NIL pair grown in Linfen (Table 2). In contrast, no significant difference was detected for TGW and GW between 7D+ and 7D− in NILs derived from NP8 family (Table 2). Collectively, these results further demonstrate that the HS2 allele in *QTgw.cau-7D* has a consistent positive effect on TGW, GW and GL.

Based on the gene annotations of Chinese Spring reference genome (https://urgi.versailles.inra.fr/download/iwgsc /IWGSC_RefSeq_Annotations/v1.0/), the *Xcau.7D-2* and *7D-ID-9* interval contains 56 high confidence genes, among

which *TraesCS7D01G107500*, *TraesCS7D01G109300* and *TraesCS7D01G110600* may be the candidate genes for *QTgw.cau-7D* (Supplementary Table S12). *TraesCS7D01G107500* and *TraesCS7D01G109300* were predicted to encode a SIP5 protein and transcription elongation factor 1, respectively. Rice grain weight gene *GW2* encodes a RING-type protein with E3 ubiquitin ligase activity (Song et al. 2007), and *TaGW2*, the ortholog of *OsGW2* in wheat, was annotated as SIP5 protein. Zheng et al. (2014) reported that a transcript elongation factor gene *TEF-7A* influenced yield-related traits in wheat. The functional description of *TraesCS7D01G110600* was a WRKY transcription factor, which is a homolog of the *Arabidopsis thaliana WRKY53*. In rice, *OsWRKY53* had been identified as a positive regulator of rice brassinosteroid (BR) signaling and played an important role in controlling growth and development (Tian et al. 2017a). Furthermore, we analyzed the expression patterns of these three genes on the wheat expVIP expression platform (http://www.wheat-expression.com/) and Wheat eFP Browser (http://bar.utoronto.ca/efp_wheat/cgi-bin/efpWe b.cgi), and found that only *TraesCS7D01G107500* and *TraesCS7D01G109300* were expressed in the developing grains. Based on the resequencing data of two parents, only one A insertion (-1985) was found in the promoter region
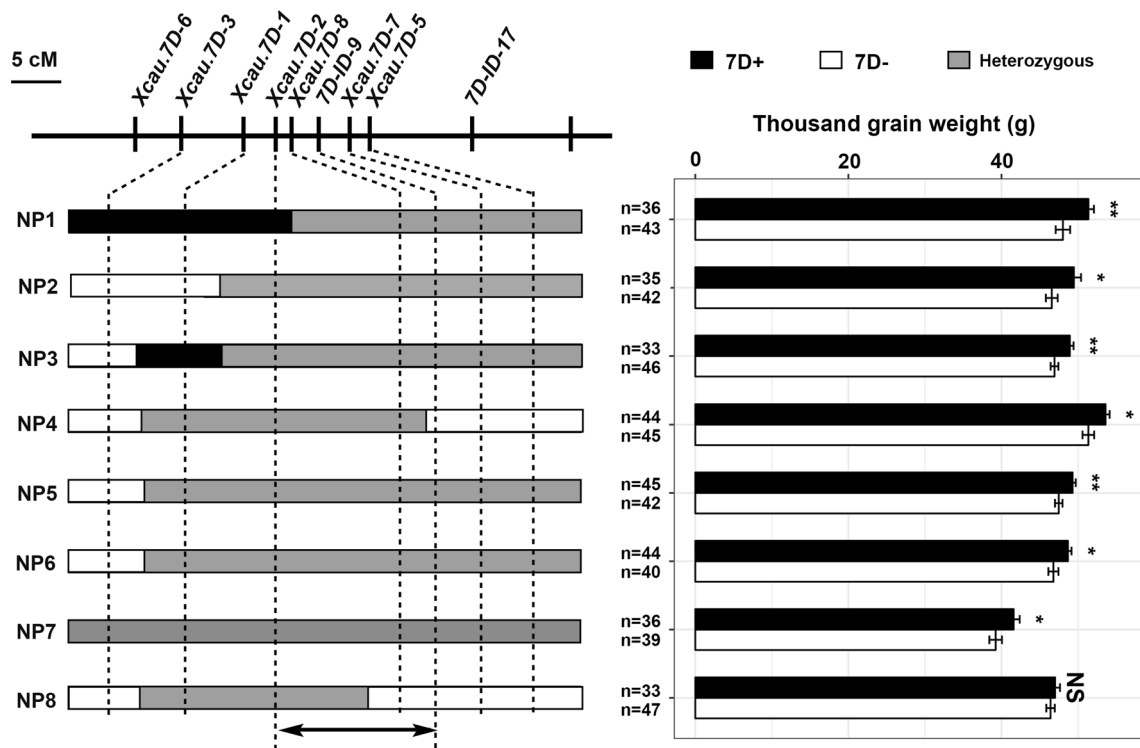
Fig. 4 Fine mapping of the *QTgw.cau-7D* locus. *Left side* is the six markers used to screen recombinants (*upside*) and the graphical genotypes of eight recombinants (*downside*). Arrows represent the 4.4 Mb interval of fine mapping. *Right side* is the comparisons of TGW between 7D+ and 7D− within each family. The values of TGW are the means (mean ± SD) of the homozygous plants in NIL families. n represents the number of homozygous plants. Black, gray and white bars represent 7D+ (HS2), heterozygous haplotypes and 7D− (4332), respectively. Significant differences are indicated by * (*P* < 0.05), ** (*P* < 0.01) (Student's *t* test)

Table 2 Mean thousand grain weight (TGW), grain length (GL) and grain weight (GW) of near isogenic lines (NILs) derived from families NP1, NP4 and NP8

Env.[a]	Genotype	NP1			NP4			NP8		
		TGW	GL	GW	TGW	GL	GW	TGW	GL	GW
E7	7D+	46.99	6.91	3.36	48.11	7.03	3.37	41.74	6.66	3.19
	7D−	40.42	6.67	3.15	40.23	6.65	3.15	40.86	6.65	3.17
	Percentage	16.25%***	3.6%***	6.67%***	19.59%****	5.71%****	6.98%****	2.15%[ns]	0.15%[ns]	0.63%[ns]
E8	7D+	45.88	6.86	3.3	47.69	7	3.33	39.2	6.72	3.08
	7D−	39.49	6.64	3.12	39.17	6.68	3.07	37.78	6.6	3.06
	Percentage	16.18%****	3.31%**	5.77%****	21.75%****	4.79%****	8.47%****	3.76%[ns]	1.82%**	0.65%[ns]
E9	7D+	45.86	6.83	3.3	48.26	6.97	3.4	42.93	6.78	3.23
	7D−	40.66	6.75	3.17	42.46	6.79	3.21	41.69	6.76	3.21
	Percentage	12.79%***	1.19%[ns]	4.1%***	13.66%****	2.65%****	5.92%****	2.97%[ns]	0.3%[ns]	0.62%[ns,b]

[a] E7, 2017–2018 Beijing; E8, 2017–2018 Hebei; E9, 2017–2018 Shanxi; [b] Percentages (%) indicate increased TGW, GL and GW in 7D+ NILs compared with 7D− NILs. ns, no significant; **P < 0.0.1; ***P < 0.001; ****P < 0.0001 (*t* test)

of *TraesCS7D01G109300* in 4332 compared with HS2 and the reference genome of CS.

Discussion

Comparisons of QTL for grain size with the previous reports

Improving grain size is a prime breeding target for wheat yield potential and end-use quality (Su et al. 2018). In the present study, a QTL analysis using the HS2/4332 RIL population revealed 23 environmentally stable QTL for grain size traits (TGW, GL and GW), which were mainly distributed on chromosome arms 1DL, 2BL, 3DS, 4BS, 5AL, 5BL, 6BL, 6DS, 7AS, 7AL and 7DS (Fig. 2, Table 1, Supplementary Table S7). QTL for grain size (TGW, GL and GW) have been found on all 21 wheat chromosomes (Chen et al. 2019). Thereby, we blasted the rough physical location using the sequences of reported markers to analyze the relationship of these QTL with our results. Due to the limited information of many previous QTL for grain size, the relationship of some identified QTL (*QGl.cau-6D1* and *QGw.cau-2B*) in this study to other reports is unclear. However, 21 of 23 stable QTL have been reported in the previous studies, which were located on chromosome arms 1DL, 2BL, 3DS, 4BS, 5AL, 5BL, 6BL, 6DS, 7AS, 7AL and 7DS (Table 1). For example, the physical position of *QGl.cau-1D-1* and *QGl.cau-1D-2* is similar to that of QTL for GL reported by Cui et al. (2016). The major stable QTL, *QGl.cau-3D1*, was consistent with the QTL identified by Wu et al. (2015). *QTgw.cau-4B-1*, *QTgw.cau-4B-2*, *QGl.cau-4B-1* and *QGw.cau-4B* were located in the genomic interval flanked by markers *wsnp_Ku_c8075_13785546* and *GENE-3134_472*. Many previous studies have demonstrated that this genomic region on chromosome 4B near the Green Revolution gene *Rht1* is associated with TGW, GW and GL (Chen et al. 2014; Guan et al. 2018; Kumar et al. 2016; Liu et al. 2014). *QTgw.cau-7A-2*, *QGw.cau-7A-2*, *QTgw.cau-7D* and *QGw.cau-7D* corresponds to the reported QTL for grain size in an RIL population crossed by an elite and a nonadapted genotype (Kumar et al. 2016). As expected, many QTL for GL and GW were co-localized with TGW, which provided valuable information about the contribution of GL and/or GW for each QTL controlling TGW. Notably, the previous studies showed that grain thickness is the most important dimension factor affecting TGW, which will be an interesting trait for analysis (Cheng et al. 2017).

Genetic relationship between PHT and grain size

Plant height is a parameter that affects lodging and grain yield in wheat breeding (Wurschum et al. 2015). However, the molecular basis of PHT in bread wheat remains largely unclear with the exception of *Rht1* and *Rht2* alleles (Dong et al. 2019). Moreover, the majority of genes that increased plant height are strongly correlated with increased grain size (Guan et al. 2018; Roder et al. 2008). The present study identified a total of seven stable QTL for PHT on six chromosomes, including *QPht.cau-2A*, *QPht.cau-3A*, *QPht.cau-3D1*, *QPht.cau-3D2*, *QPht.cau-4A*, *QPht.cau-5B* and *QPht.cau-6D1*. As expected, several QTL detected for grain size and PHT were co-localized and mainly clustered on specific regions of chromosome arms 3DS, 5BL and 6DS (Fig. 2, Table 1, Supplementary Table S7). All of the favorable alleles for these QTL were contributed by HS2, suggesting that selection of these loci that increase grain weight may also increase plant height. Notably, three co-located major QTL on chromosome arm 3DS controlling TGW, GL and PHT accounted for high phenotypic variance and exhibited stability across different environments, which are attractive targets for positional cloning. In addition, pleiotropic QTL controlling TGW and PHT have been reported in two genomic regions located on chromosome arm 4BS nearby *Rht1* and chromosome arm 7DS, respectively (Guan et al. 2018; Liu et al. 2014). For example, Jobson et al. (2018) demonstrated that NILs carrying *Rht-B1b* reduced plant height and kernel weight. Roder et al. (2008) using the BC_4F_3 introgression lines (ILs) population reported a novel plant height locus linked to grain size on chromosome arm 7DS. Interestingly, we also identified QTL controlling gain size on these two genomic regions, but no major QTL for PHT was detected, which deserves further investigation. To investigate the influence of the major QTL on chromosome arm 3DS to the other QTL, we select 88 RIL lines with the decreasing allele (4332 allele) across the interval of *QTgw.cau-3D* (*wsnp_Ex_c2258_4232538–wsnp_Ex_c18250_27065775*) for QTL analysis, and only 4 of 14 identified stable QTL were consistent with the results using 271 RIL lines, indicating that the major QTL on chromosome arm 3DS have effects on the other QTL (Supplementary Table S13).

Genetic effects of *QTgw.cau-7D*

Grain size is a complex quantitative and important agronomic trait (Cheng et al. 2015). To date, several QTL/genes for grain size have been mapped on chromosome arm 7DS. For example, Roder et al. (2008) delimited a QTL *gw1* for TGW to the interval *Xgwm295-Xgwm1002* on chromosome arm 7DS by using ILs. *TaGS-D1*, an orthologous gene of rice *OsGS3*, was mapped on chromosome arm 7DS (Zhang

et al. 2014b). Kumar et al. (2016) reported that the genomic region on chromosome arm 7DS was associated with TGW and GW. Three environmentally stable QTL for grain size were mapped on chromosome arm 7DS by Yan et al. (2017). Li and Yang (2017) placed eight grain size genes (*GS3*, *DLT*, *TGW6-7.1*, *HGW*, *TGW6-7.2*, *TGW6-7.4*, *PGL2* and *IPA1*) on the chromosome arm 7DS proximal region of *A. tauschii* by comparative genomic approach. In the present study, two stable QTL for TGW (*QTgw.cau-7D*) and GW (*QGw.cau-7D*) in the same interval of 7DS were identified and were narrowed down to the 4.4 Mb physical interval using NILs (Fig. 3, Table 1, Supplementary Table S7, Fig. 4). The positive allele of *QTgw.cau-7D* increased TGW by 12.79–21.75%, and the effect was mainly attributed to the primary effect on grain width (Table 2). When compared with the previous results, the physical location of our QTL was consistent with QTL region of *gw1* reported by Roder et al. (2008). However, the effect of *gw1* on TGW was significantly and positively correlated with grain length and plant height, which was obviously different from our study. Overall, we proposed that *QTgw.cau-7D* may be of great value in molecular marker-assisted selective breeding programs.

Candidate genes for QTL controlling TGW, GL, GW and/or PHT

To date, a number of wheat genes for TGW, GL or GW have been identified using a homology-based approach, such as *TaCwi*, *TaCYP78A3*, *TaGS5-3A* and *TaGL3* (Jiang et al. 2015; Ma et al. 2015, 2016; Yang et al. 2019). In addition, some dwarfing genes have been characterized in wheat (McIntosh et al. 2017; Peng et al. 1999). To identify the candidate genes for TGW, GL, GW and/or PHT, we analyzed the annotated genes within the peak regions of detected QTL in the present study (Supplementary Table S14). The gene *TraesCS4B01G043100* (*Rht1*) was located in the peak region of *QTgw.cau-4B-1*, *QGl.cau-4B-2* and *QGw.cau-4B*, which was associated with wheat yield component traits in the previous studies (Flintham et al. 1997; Guan et al. 2018). *OsSPL16* belongs to the SBP domain family of transcription factors, which encodes a protein that is a positive regulator of cell proliferation (Wang et al. 2012b). *QGl.cau-5B* and *QPht.cau-5B* were co-located in the genomic region of chromosome 5B flanked by markers *GENE-3145_39* and *Ku_c1083_131*4, among which *TraesCS5B01G265600* encoded a squamosa promoter-binding-like protein homologous to SPL9 of *Arabidopsis*. The QTL region controlling GL on chromosome arm 7AS contained an orthologous gene (*TraesCS7A01G154900*) to Mitogen-activated protein kinase (MAPK) kinase 4 (*MKK4*) in *Arabidopsis*. MAPK cascades have been identified as a factor for grain size in rice (Duan et al. 2014; Guo et al. 2018; Liu et al. 2015).

Up to now, multiple major QTL for TGW were identified in different genetic backgrounds (Cui et al. 2014; Gegas et al. 2010; Kato et al. 2000; Kuchel et al. 2007; Quarrie et al. 2006; Wang et al. 2009). However, these QTL were defined in a relatively large chromosome interval due to the limited numbers of markers (Su et al. 2018) or lack of recombination events within the targeted QTL region in a finite population size (Raihan et al. 2016). Therefore, many researchers have tried to employ a fine-mapping approach to validate the QTL or narrow the genomic interval within the targeted QTL region (Brinton et al. 2017; Huang et al. 2015; Roder et al. 2008). In the present study, the *QTgw.cau-7D* was delimited to the 4.4 Mb physical interval with 56 annotated genes, among which *TraesCS7D01G107500*, *TraesCS7D01G109300* and *TraesCS7D01G110600* may be the candidate genes for *QTgw.cau-7D* (Supplementary S12).

Compliance with ethical standards

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

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