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Identification and validation of stable quantitative trait loci for yield component traits in wheat

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ABSTRACT

Grain weight and grain number are important yield component traits in wheat and identification of underlying genetic loci is helpful for improving yield. Here, we identified eight stable quantitative trait loci (QTL) for yield component traits, including five loci for thousand grain weight (TGW) and three for grain number per spike (GNS) in a recombinant inbred line population derived from cross Yangxiaomai/Zhongyou 9507 across four environments. Since grain size is a major determinant of grain weight, we also mapped QTL for grain length (GL) and grain width (GW). *QTGW.caas-2D*, *QTGW.caas-3B*, *QTGW.caas-5A* and *QTGW.caas-7A.2* for TGW co-located with those for grain size. *QTGW.caas-2D* also had a consistent genetic position with *QGNS.caas-2D*, suggesting that the pleiotropic locus is a modulator of trade-off effect between TGW and GNS. Sequencing and linkage mapping showed that *TaGL3-5A* and *WAPO-A1* were candidate genes of *QTGW.caas-5A* and *QTGW.caas-7A.2*, respectively. We developed Kompetitive allele specific PCR (KASP) markers linked with the stable QTL for yield component traits and validated their genetic effects in a diverse panel of wheat cultivars from the Huang-Huai River Valley region. KASP-based genotyping analysis further revealed that the superior alleles of all stable QTL for TGW but not GNS were subject to positive selection, indicating that yield improvement in the region largely depends on increased TGW. Comparative analyses with previous studies showed that most of the QTL could be detected in different genetic backgrounds, and *QTGW.caas-7A.1* is likely a new QTL. These findings provide not only valuable genetic information for yield improvement but also useful tools for marker-assisted selection.

1. Introduction

Wheat (*Triticum aestivum* L.) is a staple food for approximately-one-third of the world population [1]. It is predicted that agricultural production must increase at least 60% to meet the increasing population by 2050 (https://www.fao.org/faostat/en/). Therefore, improved yield is an essential breeding target to ensure food security. Mining genetic loci for yield-related traits will be helpful to support breeding programs aimed at achieving these objectives.

Wheat grain yield is determined by three yield component traits, viz. thousand grain weight (TGW), grain number per spike (GNS) and spike number (SN) per unit area. Quite a few genes associated with yield components have been identified by homology-based cloning using comparative genomics [2]. For example, *TaGL3-5A*, orthologous to rice (*Oryza sativa* L.) *GL3*, improves TGW by increasing grain length (GL) [3]. *GL3* is known to encode a protein phosphatase kelch (PPKL) family and control grain size by regulating Cyclin-T1-3 [4]. *WAPO-A1* isolated by map-based cloning is a casual gene affecting spikelet number per spike [5,6]. *WAPO1* controls number of spikelets by regulating the timing of terminal spikelet formation in wheat [6]. *GNI-A1* was cloned for grain number per spikelet using a population of recombinant inbred substitution lines [7]. *GNI1* encodes a homeodomain leucine zipper class I (HD-Zip I) transcription factor and is identified as responsible for increasing floret fertility in wheat [7]. *TB1* regulates inflorescence architecture and development in wheat [8]. *GW2* encodes a RING-type E3 ubiquitin ligase and negatively regulates grain size [9,10]. *GS5* encodes a serine carboxypeptidase and positively regulates grain size [11,12].

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With rapid advances in wheat genome sequencing and high-throughput genotyping, a large number of genetic loci for yield component traits have been identified in wheat through linkage mapping and association analysis [13–20]. Among them, many stable genetic loci for yield components were defined using genome-wide meta-analyses, providing genetic targets for gene cloning and molecular marker-assisted breeding [2].

The Chinese landrace Yangxiaomai (YXM) and an elite wheat cultivar Zhongyou 9507 (ZY9507) have large differences in pre-harvest sprouting (PHS). We recently identified QTL for PHS using a recombinant inbred line (RIL) population from the cross YXM/ZY9507 [21]. YXM and ZY9507 also differ in spike and grain morphology, so the RIL population is suitable to mine genetic loci for yield component traits. The objectives of this study were to (1) identify stable major QTL for TGW and GNS in the RIL population, (2) develop breeding-applicable markers closely linked with the QTL, and (3) validate genetic effects of the QTL in a panel of 166 elite cultivars.

2. Materials and methods

2.1. Plant materials, field trials and phenotypic investigations

A total of 194 RILs derived from the cross YXM/ZY9507 was used for QTL mapping. Field trials were performed at Gaoyi (Hebei province) during the 2018–2019 and 2019–2020 cropping seasons, and Xinxiang (Henan) during 2019–2020 and 2020–2021. The RILs were planted in randomized complete blocks with three replications. One row per block was planted for each genotype. Each row was 1 m long with 30 seeds sown. Twenty randomly selected spikes in each row were harvested and manually threshed. All grains obtained from 20 spikes were used to measure GL, GW and TGW. Grain length (GL), grain width (GW), TGW and GNS were scored using Wanshen SC-G seed detector (Hangzhou Wanshen Detection Technology Co., Ltd., Hangzhou, Zhejiang, China).

A panel of 166 wheat cultivars from the Huang-Huai River Valley region, the largest wheat-producing region in China, were used to validate genetic effects of the QTL of interest, and the phenotypic data are available in Li et al. [22].

2.2. Genotyping, map construction and QTL detection

The YXM/ZY9507 RIL population and parents were previously genotyped using 15 K single nucleotide polymorphism (SNP) chips. A genetic linkage map constructed includes 1702 bin markers, spanning 2630.9 cM on 21 wheat chromosomes. The average length of linkage groups was 125.3 cM with an average marker interval of 1.6 cM [21]. This genetic map was used to map QTL with the composite interval mapping (CIM) in Windows QTL Cartographer Version 2.5 [23]. Walking steps, control markers and window size were set as 1, 5 and 10 cM, respectively. The logarithm of odds (LOD) was calculated based on 1000 random permutation tests at $P < 0.05$. Significant QTL were declared when the LOD values were more than 2.5. Genetic maps of QTL were drawn using MapChart v2.3 [24]. Multi-environment QTL analysis also was conducted to confirm the stable QTL using "met" function in QTL IciMapping V4.2 (https://isbreeding.caas.cn/rj/qtllcmapping/294445.htm).

2.3. Marker development and QTL validation

The SNPs within target QTL were used to develop KASP markers using PolyMarker (http://www.polymarker.info/) [25]. KASP genotyping was performed following the protocol in Dong et al. [26]. The genetic effects of QTL were validated by association analyses in a panel of 166 cultivars from the Huang-Huai River Valley wheat region.

2.4. Cloning and sequence analysis of genes WAPO-A1 and GNI-D1

Gene-specific primers were designed using the software SnapGene (https://www.snapgene.com/) and synthesized by Shanghai Sangon Biotech Co., Ltd. (https://www.sangon.com/). All PCR primers used in this study were described in Table S1. PCR was performed in a total volume of 15 μL, including 1.5 μL genomic DNA (100 ng μL^{-1}), 7.5 μL 2× *Taq* Mix, 1 μL forward primer (10 μmol μL^{-1}), 1 μL reverse primer (10 μmol μL^{-1}) and 4 μL ddH_2O. Reaction conditions were 95 °C for 5 min, followed by 37 cycles of 95 °C for 45 s, annealing at 60 °C for 30 s, and 72 °C for 1 min, with a final extension of 72 °C for 5 min. PCR products were sequenced by Shanghai Sangon Biotech Co., Ltd. (https://www.sangon.com/), and the difference in sequences between YXM and ZY9507 was determined.

2.5. Statistical analysis

Analysis of variance (ANOVA) was performed using PROC GLM in SAS 9.4 (SAS Institute Inc., Cary, NC, USA), where genotypes, environments, replicates and genotype × environment interactions were considered as random effects. The best linear unbiased prediction (BLUP) of each trait was calculated by combining PROC GLM and PROC MIXED in SAS 9.4. Correlation analyses and the Student's *t*-tests were conducted by PROC CORR and PROC TTEST, respectively, in SAS 9.4.

Broad-sense heritability (H^2) was calculated using the formula: $H^2 = \sigma_g^2/(\sigma_g^2 + \sigma_{ge}^2/e + \sigma_\varepsilon^2/re)$, where σ_g^2, σ_{ge}^2 and σ_ε^2 represent the phenotypic variances due to genotypes, genotype-environment interactions and residual errors, respectively; r is the number of replicates and e is the number of environments [27].

3. Results

3.1. Phenotypic evaluation

Grain length (GL), grain width (GW), thousand grain weight (TGW) and grain number per spike (GNS) in YXM, ZY9507 and the RILs were phenotyped in four environments. Significant differences ($P < 0.01$) in these traits were detected between YXM and ZY9507 (Table S2). On average, ZY9507 increased GL, GW and TGW by 1.68 mm, 0.72 mm and 25.57 g, respectively, and decreased GNS by 9.63 compared with YXM (Table S2). There were wide phenotypic variations in GL (5.31 to 7.40 mm), GW (2.43 to 3.38 mm), TGW (23.60 to 48.37 g) and GNS (21.00 to 56.00) across the RIL population (Table S2). The phenotypic scores for each trait in each environment were normally distributed, indicating the traits were quantitatively inherited (Fig. S1). The correlation coefficients for each trait among environments were significant ($P < 0.05$) (Table S3). Significantly positive correlations were detected between grain size (GL and GW) and TGW, with correlation coefficients ranging from 0.51 to 0.92 ($P < 0.01$) across environments (Table S4). GL, GW, TGW and GNS had high H^2 of 0.94, 0.88, 0.90, and 0.84, respectively, although genotype (G), environment (E) and G × E interaction had significant effects on each trait (Tables S2, S5). These results show that the tested traits are largely controlled by genetic factors in the RIL population.

3.2. QTL mapping

Twenty-four QTL were identified based on the phenotypic scores of the traits across the four environments, including ten

for GL on chromosomes 2A, 4A, 5A (2), 5D, 6D (2), 7A (2) and 7D, six for GW on chromosomes 1A, 2A, 2D, 3B, 5A and 7A, five for TGW on chromosomes 2D, 3B, 5A and 7A (2), and three for GNS on chromosomes 2D, 4A and 6B (Fig. 1; Table S6). Among them, twenty-two QTL were detected in two or more environments and the BLUP datasets, and thus were regarded as stable QTL

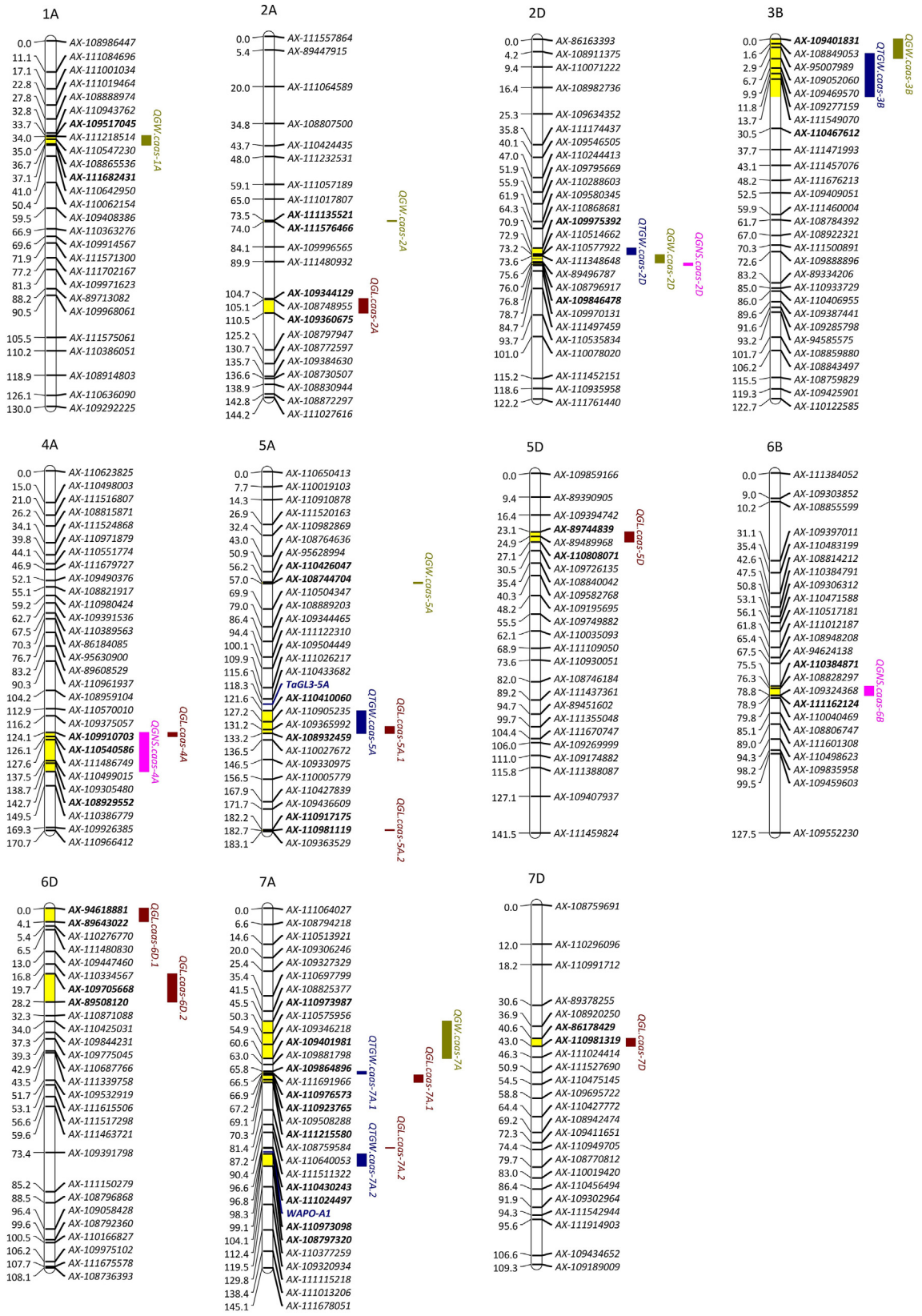

Fig. 1. Genetic mapping of QTL for GL, GW, TGW and GNS in the Yangxiaomai/Zhongyou 9507 RIL population. Target regions of the QTL are labeled with yellow bars; gene-specific markers are shown in blue; flanking markers of target QTL are shown in bold. QTL, quantitative trait locus; GL, grain length; GW, grain width; TGW, thousand grain weight; GNS, grain number per spike; RIL, recombinant inbred line.

(Table S6). We also conducted multi-environment QTL analysis. Except *QGL.caas-6D.2* and *QGL.caas-7A.1* for grain length, all the stable QTL could be detected in multi-environmental trials (Table S7).

3.2.1. Stable QTL for grain size

Nine stable QTL for GL were detected, and ZY9507 alleles at all loci conferred positive effects (Table S6). Among them, three QTL, *QGL.caas-5D*, *QGL.caas-5A.2* and *QGL.caas-7A.1* were identified in all environments, explaining 11.6%–14.4%, 5.8%–11.1% and 4.6%–10.0% of the respective phenotypic variances. *QGL.caas-6D.2* was detected in three environments and BLUP values, accounting for 4.7%–6.4% of the phenotypic variances. The other five QTL, *QGL.caas-2A*, *QGL.caas-5A.1*, *QGL.caas-6D.1*, *QGL.caas-7A.2*, and *QGL.caas-7D* were detected in two environments and BLUP values, contributing 3.5%–6.4%, 3.4%–4.8%, 4.7%–4.9%, 3.6%–4.3% and 6.0%–10.9% of the phenotypic variances, respectively.

Five stable QTL for GW were identified and the alleles in ZY9507 conferred higher GW (Table S6). *QGW.caas-2D* and *QGW.caas-3B* were detected in three environments and BLUP values and contributed 9.8%–11.4% and 3.6%–7.7% of the phenotypic variances, respectively. *QGW.caas-1A*, *QGW.caas-5A* and *QGW.caas-7A*, were detected in two environments and BLUP values, explaining 5.5%–7.6%, 4.8%–10.3% and 6.0%–11.5% of the respective phenotypic variances.

3.2.2. Stable QTL for TGW

Five stable QTL for TGW were identified and the alleles from ZY9507 conferred higher TGW (Table S6). *QTGW.caas-2D* was detected in all environments, explaining 5.4%–11.4% of the phenotypic variances. *QTGW.caas-7A.1* was detected in three environments and BLUP values, accounting for 4.8%–13.8% of the phenotypic variances. Three other QTL, *QTGW.caas-3B*, *QTGW.caas-5A* and *QTGW.caas-7A.2* were detected in two environments and BLUP values, accounting for 5.2%–7.5%, 7.3%–13.1% and 5.9%–9.5% of the phenotypic variances, respectively. *QTGW.caas-2D*, *QTGW.caas-3B, QTGW.caas-5A* and *QTGW.caas-7A.2* shared the same genetic positions as *QGW.caas-2D*, *QGW.caas-3B*, *QGL.caas-5A.1* and *QGL.caas-7A.2*, respectively. Considering that grain size is a major determinant of grain weight, the four QTL for grain weight can be contributed by the co-located QTL for grain size.

3.2.3. Stable QTL for GNS

Three stable QTL for GNS were mapped on chromosomes 2D, 4A and 6B, respectively, and YXM alleles conferred higher GNS (Table S6). *QGNS.caas-2D* and *QGNS.caas-4A* were identified in all four environments and BLUP values, explaining 9.8%–21.5% and 5.3%–12.5% of the phenotypic variances, respectively. *QGNS.caas-6B* were detected in two environments and BLUP values and accounted for 5.2%–7.3% of the phenotypic variances. Interestingly, *QGNS.caas-2D* was co-located with *QTGW.caas-2D* and *QGW.caas-2D*, suggesting a trade-off between grain number and grain weight or size.

3.3. Genetic evaluation for the QTL with yield-related genes

To identify candidate genes for the above QTL, we investigated annotated genes in the target regions according to Chinese Spring reference genome. The target intervals of the three QTL clusters contained or were adjacent to yield-related genes previously reported. *QTGW.caas-5A* and *QGL.caas-5A.1* were co-localized between *AX-110410060* and *AX-108932459* in the interval 574.4–598.0 Mb on chromosome 5A (Table S6). *TaGL3-5A*, a gene associated with grain size, was previously located at ~571.8 Mb on chromosome 5A [3]. We genotyped the YXM/ZY9507 RIL population to investigate the genetic relationship between *TaGL3-5A* and *QTGW.caas-5A* using the previously reported KASP marker of *TaGL3-5A* (Table S1) [3]. Linkage analysis indicated that *TaGL3-5A* was very close to *QTGW.caas-5A* (Fig. 1), suggesting that *TaGL3-5A* was a candidate gene for *QTGW.caas-5A* and *QGL.caas-5A.1*.

QTGW.caas-7A.2 and *QGL.caas-7A.2* were co-localized between *AX-110430243* and *AX-108797320* in the interval 670.9–683.1 Mb on chromosome 7A (Table S6). *WAPO-A1* is located at the ~674.1 Mb on this chromosome and acts as an important regulator of spikelet number per spike [5,6]. We detected two SNPs between the parents in the promoter region of *WAPO-A1* and converted them into KASP markers (*KASP1_WAPO-A1* and *KASP2_WAPO-A1*) (Table S1). The two KASP markers had the same genotypes in the RIL population and mapped *WAPO-A1* in the target region of *QTGW.caas-7A.2* and *QGL.caas-7A.2* (Fig. 1). These results indicate that *WAPO-A1* is likely the causal gene of *QTGW.caas-7A.2* and *QGL.caas-7A.2*.

QGW.caas-2D, *QTGW.caas-2D* and *QGNS.caas-2D* were co-localized between markers *AX-109975392* and *AX-109846478* on chromosome 2D, spanning the interval of 475.0–508.1 Mb (Table S6). *GNI-D1*, at ~490.1 Mb on chromosome 2D, is an ortholog of *GNI-A1*, a regulator of trade-off between grain number and grain weight in tetraploid wheat [28]. We sequenced *GNI-D1* but found no polymorphic site in the 2-kb promoter region and exons between YXM and ZY9507 (Table S1). In addition, an effect of *GNI1* on grain size was not reported in previous studies [7,28]. Therefore, *GNI-D1* was probably not a candidate gene in *QTGW.caas-2D*.

3.4. Validation of the major QTL for TGW and GNS

We developed closely linked KASP markers to further validate genetic effects of the stable QTL for yield component traits (Tables S1, S8). Genotyping showed that cultivars carrying the ZY9507 allele at *QTGW.caas-2D* (co-located with *QGNS.caas-2D*) increased TGW by 3.8%–8.7%, but decreased GNS by 10.6%–11.7% in the diversity panel of wheat cultivars from the Huang-Huai River Valley region (Fig. 2a, d). The ZY9507 allele was present in 92.7% of panel members, suggesting that it had been subjected to positive selection in wheat breeding. The ZY9507 alleles at *QTGW.caas-3B* and *QTGW.caas-7A.1* also occurred at high frequencies (84.0% and 83.9%, respectively) and conferred higher TGW (Fig. 2b, c). The YXM alleles at *QGNS.caas-4A* and *QGNS.caas-6B* conferred higher GNS but were present at lower frequencies (48.5% and 40.9%, respectively) than the contrasting ZY9507 alleles (Fig. 2e, f). Overall, stable QTL, except *QGNS.caas-6B*, had significant effects on the target traits.

4. Discussion

4.1. Potential application of the QTL for yield component traits in wheat breeding

Eight stable QTL for TGW (*QTGW.caas-2D*, *QTGW.caas-3B*, *QTGW.caas-5A*, *QTGW.caas-7A.1* and *QTGW.caas-7A.2*) or GNS (*QGNS.caas-2D*, *QGNS.caas-4A* and *QGNS.caas-6B*) were identified in two or more environments and the corresponding BLUP data. All were major QTL accounting for approximately 10% of the phenotypic variances (Table S6). Among them, *QTGW.caas-2D*, *QTGW.caas-3B*, *QTGW.caas-5A* and *QTGW.caas-7A.2* had consistent genetic positions with the QTL for grain size. Given that grain size is a major determinant of grain weight, the co-location of the QTL for grain weight and grain size demonstrated their reliability. Likewise, *QTGW.caas-2D* and *QGNS.caas-2D* were also co-located and explained up to 11.4% and 21.5% of phenotypic variances, respectively, suggesting that the pleiotropic genetic locus is a major modulator of the trade-off effect between grain number

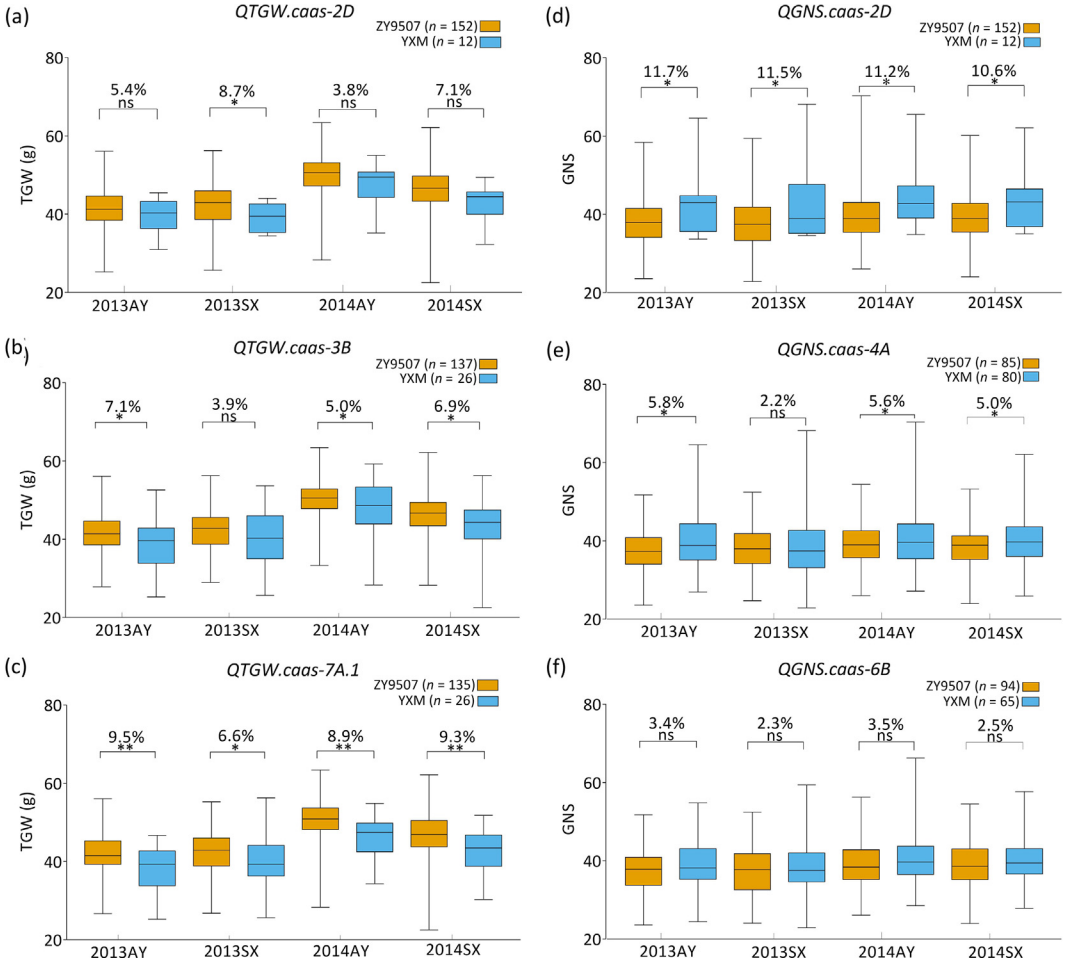

Fig. 2. Validation of QTL: *QTGW.caas-2D* (a), *QTGW.caas-3B* (b), *QTGW.caas-7A.1* (c), *QGNS.caas-2D* (d), *QGNS.caas-4A* (e), and *QGNS.caas-4A* (f) in the panel of 166 wheat cultivars from the Huang-Huai River Valley region. *, $P < 0.05$; **, $P < 0.01$; ns, not significant; 2013, 2012–2013 cropping season; 2014, 2013–2014 cropping season; AY, Anyang; SX, Suixi; QTL, quantitative trait locus; TGW, thousand grain weight; GNS, grain number per spike.

and grain weight. Most importantly, association analyses showed that the majority of QTL had significant effects on the target traits in a diverse panel of elite cultivars. The superior QTL alleles for TGW had undergone positive selection in the Huang-Huai River Valley wheat region where grain weight is considered the most important factor contributing to yield improvement [29]. By contrast, superior alleles of the QTL for GNS had not been subjected to positive selection. Therefore, these QTL for GNS probably have greater application potential in wheat breeding. Combinational analysis showed that the five stable QTL for TGW had significant additive effects, increasing TGW by 28.3% (~9 g) in the RIL population (Table S9; Fig. S2). Likewise, pyramiding the three QTL for GNS also had significant additive effects and increased GNS by 20.8% (~6) (Table S9; Fig. S2). In summary, we mined a few useful QTL for yield improvement and developed breeding-applicable tools for marker-assisted selection in wheat breeding.

4.2. Comparisons of stable QTL for TGW or GNS with those previously reported

We identified stable QTL for TGW or GNS and predicted that *TaGL3-5A* and *WAPO-A1* were candidate genes for *QTGW.caas-5A* and *QTGW.caas-7A.2*, respectively, based on results from sequencing and genetic mapping (Fig. 1). *QTGW.caas-2D* and *QGNS.caas-2D* were co-localized between markers *AX-109975392* and *AX-109846478* on chromosome 2D, spanning the interval 475.0–508.1 Mb (Table S6). A QTL for TGW was mapped between *IWA4789* and *IWB53594*, spanning the region of 481.6–523.2 Mb and overlapping with the target interval of *QTGW.caas-2D* and *QGNS.caas-2D* [30]. *QTGW.caas-3B* was mapped between *AX-109401831* and *AX-110467612* in the interval 8.5–38.1 Mb on chromosome 3B (Table S6). A SNP marker *IWB40900* was associated with TGW and near to *QTGW.caas-3B* [15]. *QGNS.caas-4A* in the interval of 658.9–683.8 Mb is located in a QTL-rich cluster [2]. *QGNS.caas-6B* was identified in the interval of 675.4–680.9 Mb. *IWA1679* associated with *GNS* is close to *QGNS.caas-6B* [31]. Overall, the above QTL have consistent locations with those previously reported, suggesting that they can be detected in different genetic backgrounds. These findings further showed that the QTL were stable and valuable genetic loci for wheat yield improvement. *QTGW.caas-7A.1* was flanked by the markers *AX-109864896* and *AX-110976573* within the interval 241.4–270.4 Mb on chromosome 7A (Fig. 1; Table S6). No yield-related gene or QTL was previously identified in the target interval of *QTGW.caas-7A.1*, indicating that this QTL likely represents a new locus for TGW.

CRediT authorship contribution statement

Lingli Li and **Shuanghe Cao:** wrote the draft manuscript. **Lingli Li:** performed the experiments. **Yingjie Bian, Yan Dong, Jie Song, Dan Liu, Jianqi Zeng** and **Fengju Wang:** participated in field trials. **Shuanghe Cao** and **Yan Zhang:** designed the experiment. **Xian-**

chun Xia, **Yong Zhang** and **Zhonghu He:** assisted in writing the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data for this article can be found online at https://doi.org/10.1016/j.cj.2022.09.012.

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