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ORIGINAL ARTICLE

Characterization of three wheat grain weight QTLs that differentially affect kernel dimensions

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Abstract

Key message **The *QGw.nau-2D*, *QGw.nau-4B* and *QGw.nau-5A* intervals were investigated for their effects on weight, length, width, and thickness of kernels and their differential roles in determining kernel size and shape were demonstrated.**

Abstract Grain weight (GW) contributes greatly to wheat yield and is directly related to kernel size and shape. Although over 100 quantitative trait loci (QTLs) for GW have been reported in the literatures, few have been well characterized for their association with kernel traits. In this study, three GW QTLs identified in elite cultivar 'Nanda2419' ('Mentana'), including *QGw.nau-2D*, *QGw.nau-4B* and *QGw.nau-5A*, were investigated through near isogenic line (NIL) development and evaluation. NILs for all three QTLs and one NIL with both *QGw.nau-4B* and *QGw.nau-5A* were developed with the help of marker-assisted selection after two to three generations of backcross using cultivar 'Wangshuibai' as the recurrent parent. One NIL with *QGw.nau-4B* in the background of cultivar 'Wenmai6' was also obtained. In four different field trials, these NILs consistently produced heavier kernels than the recurrent parents. *QGw.nau-4B* showed the largest effect on GW; its presence resulted in 0.4–0.5 g increase of hundred-grain weight, depending on genetic backgrounds. *QGw.nau-4B* and *QGw.nau-5A* functioned additively in conditioning GW. These three QTL intervals showed pleiotropic effects on, or close linkage with genes for, spike length, plant height and flag leaf width, respectively, and acted differentially in determining the kernel dimensions that are the major GW determinants. They all conditioned wider kernels with *QGw.nau-5A* displaying the largest effect. *QGw.nau-4B* and *QGw.nau-5A* also conditioned thicker kernels but had opposite effects on kernel length. This study demonstrated that marker-assisted selection is effective for GW improvement. The availability of GW NILs could facilitate cloning of GW genes and unraveling of kernel development mechanisms.

Introduction

Wheat (*Triticum aestivum* L.), as one of the world's major staple crops, feeds approximately 40 % of the world's population (Gupta et al. 2008). Wheat yield has greatly increased since the introduction of the 'Green Revolution' *Rht* genes; however, development of new varieties with higher yield potential is still the focus of worldwide wheat breeding programs because of the increasing global demand for wheat due to faster population growth.

The product of grain weight (GW), kernel number per spike and number of fertile spikes per unit area determines the unit wheat yield. These three yield components are physiologically related and affected greatly by environmental factors. Since GW has the highest heritability relative to the other two yield components, it is more suitable for early selection in breeding (Xiao et al. 2012). In China and other countries, GW increase is evident among cultivars developed in the last century (Calderini et al. 1995; Zhou et al. 2007; Underdahl et al. 2008). GW is directly related to kernel size and shape (KSS), which is mainly determined by

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Table 1 Target QTLs intervals and the linked markers for forward selection

Target QTL	Interval length (cM)	Linked markers listed in mapping order[a]	References
QGw.nau-2D	4	GWM261-WMC112/CFD53-GPW4080	Wu et al. (2014)
QGw.nau-4B	1.3	GWM495-GWM149-WMC349	Xue et al. (2008)
QGw.nau-5A	13.4	MAG1281-BARC56-WMC96-BARC100-GWM186	Xue et al. (2008)

[a] '/' indicates co-segregating markers

kernel length (KL), kernel width (KW) and kernel thickness (KT) (Campbell et al. 1999; Dholakia et al. 2003; Breseghello and Sorrells 2006; Sun et al. 2009). Larger kernels usually contribute to higher GW and flour yield as well as better seedling vigor (Blair 1992; Chastain et al. 1995; Botwright et al. 2002). Understanding the genetic control of GW would help us further raise the wheat yield potential.

With the help of molecular markers, more than 100 quantitative trait loci (QTLs) for GW in wheat have been identified to date using bi-parental populations or cultivar populations (Ammiraju et al. 2001; Groos et al. 2003; Huang et al. 2003, 2004, 2006; Quarrie et al. 2005; Breseghello and Sorrells 2007; Cuthbert et al. 2008; Zhang et al. 2008; Sun et al. 2009, 2010; Zheng et al. 2010; Mir et al. 2012; Rustgi et al. 2013; Maphosa et al. 2014; Tyagi et al. 2014). These QTLs distribute on all 21 chromosomes of the wheat genome. Some of these QTLs were detected in multiple studies and verified in meta-analysis (Tyagi et al. 2014); a few have been validated in association mapping (Breseghello and Sorrells. 2006; Reif et al. 2010; Neumann et al. 2011; Mir et al. 2012; Wang et al. 2012a) or using introgression lines or near isogenic lines (NILs) (Röder et al. 2008; Simmonds et al. 2014). Due to the high level of homogeneity, NILs are widely used in validation, fine mapping and isolation of various QTLs to reduce the background noise (Röder et al. 2008; Wang et al. 2010; Deng et al. 2011; Xue et al. 2013; Wu et al. 2014).

QTL or genes conditioning GW are always associated with one or more KSS factors due to their causal relationship. The characterized genes for rice GW, such as, *GS3*, *GS5*, *GW2, qSW5/GW5* and *OsSPL16/GW8*, all showed affects on one or more KSS factors (Fan et al. 2006; Li et al. 2011; Song et al. 2007; Shomura et al. 2008; Wan et al. 2008; Wang et al. 2012b). Wheat *TaGW2*, the ortholog of *OsGW2*, is also associated with KW, KT and roundness (Su et al. 2011; Maphosa et al. 2014). Moreover, a few QTL clusters in the wheat genome that are related to GW as well as KSS factors were also identified. These included intervals on chromosomes 2A, 2B, 2D, 4B, 5D, and 6A for GW and KW (Sun et al. 2009; Ramya et al. 2010), the interval on chromosome 5B for GW and KL (Ramya et al. 2010), and intervals on chromosomes 2A, 5A, 5B, 6A and 7A for GW and kernel diameter (Sun et al. 2010; Tsilo et al. 2010). Thus, dissection of the KSS factors is important for us to understand the mechanisms underlying GW formation.

In the RIL population derived from 'Nanda2419' ('Mentana') × 'Wangshuibai' (WSB) developed in our laboratory, five QTLs associated with GW, distributed on 2D, 3A, 4B, 4D and 5A, were identified (Jia et al. 2013; unpublished data). All these QTLs increase GW through the Nanda2419 alleles and did not show epistasis (Jia et al. 2013). Of them, *QGw.nau-4B* and *QGw.nau-5A* were detected in all trials with the LOD score larger than 5 in five of six cases and explained up to 30.5 % phenotypic variation. *QGw.nau-2D* was a QTL with minor effects and the corresponding interval was also related to spike length (Wu et al. 2014). In this study, we report the development of NILs for these three QTLs and the subsequent characterization of their effects on GW and kernel dimensions.

Materials and methods

Plant materials

Local variety WSB and improved cultivar 'Wenmai6' (W6) were chosen as recurrent parents for NIL development. WSB is known for its scab resistance but poor in agronomic performance. W6 was a high-yielding cultivar developed in Henan province in 1990s. In the local trials, W6 had a thousand GW 4 g higher than Nanda2419 that in turn was 6 g higher than WSB in GW. Due to lack of polymorphism in the *QGw.nau-2D* and *QGw.nau-5A* intervals between W6 and Nanda2419, W6 was only employed in the *QGw.nau-4B* NIL development, in which Nanda2419 was used as the QTL donor. When WSB was employed as the recurrent parent, three lines (NW23, NW70, NW53) with higher WSB genetic composition from the Nanda2419 × WSB RIL population were used as the donor parents.

Marker-assisted NIL selection

NILs were selected solely based on marker-assisted selection. Markers used in marker-assisted selection of *QGw.nau-2D*, *QGw.nau-4B* and *QGw.nau-5A* intervals are listed in Table 1. At each backcross generation, plants were genotyped with the two flanking markers of the target QTL intervals. For marker-assisted background selection (BS), starting at BC_2, plants with the target QTLs were genotyped with non-linked SSR markers to roughly estimate the proportions of the recurrent parent genetic composition (RGC %) (Xue et al. 2010). In the crosses of WSB with the NW lines, markers polymorphic between the parents based on the genome marker map constructed using the Nanda2419 × WSB RIL population by Xue et al. (2008) were used for BS. In the cross of W6 with Nand2419, about two markers per chromosome arm that were polymorphic between the parents were identified for BS by surveying the parents. The BCF_1 plants harboring the target QTLs and with the highest RGC % were selected for further backcross. At BC_2F_1 or BC_3F_1 generation, the selected plants were selfed. Plants homozygous at the target intervals were then identified in the selfed progenies using all the markers mapping to the QTL intervals (Table 1) and surveyed again with the remaining polymorphic background markers. The RGC % of the NILs was estimated based on survey of all the polymorphic markers.

Marker genotyping

Total genomic DNA was extracted from young leaves according to Ma and Sorrells (1995). The PCR was performed in a PE9600 or 9700 thermal cycler (PerkinElmer, Norwalk, CT, USA) in a volume of 10 μl containing 10–20 ng of template, 2 pmol of each of the primers, 2 nmol of each of the dNTPs, 15 nmol of $MgCl_2$, 0.1 U Taq DNA polymerase and 1 X PCR buffer. The PCR profile was as follows: one cycle of 94 °C 3 min, followed by 35 cycles of 94 °C 30 s, 50–60 °C (depending on the specific primers) 30 s and 72 °C 40 s, and a final extension at 72 °C for 5 min. The PCR products were separated in 8 % non-denaturing poly-acrylamide gels with a 19:1 or 39:1 acrylamide/bisacrylamide ratio, using a constant voltage of 180 V for 2–3 h, and then silver stained as described by Bassam et al. (1991).

Phenotyping

Parents and NILs were evaluated in 2012–2014 seasons in a field of Jiangpu (JP, latitude 32°02′N, longitude 118°37′E) experimental station of Nanjing Agricultural University (NAU), Jiangsu, and a field at Fengyang county (FY, latitude 32°52′N, longitude 117°33′E), Anhui. The trials were performed using a randomized complete block design with two replicates. Each plot included two 1.5-m rows spaced by 0.25 m. Fifteen seeds were planted per row. Commonly undertaken cultivation practices in wheat production were applied to these trials.

Ten plants chosen from the middle of each plot were surveyed for plant height (PH), total tiller number per plant (TN), spike length (SPL), spikelet number per spike (SPN), flag leaf length (FLL), flag leaf width (FLW). PH was measured from the soil surface to the top of the main spike excluding the awn at physiological maturity. SPL of the main tiller was measured in centimeters from the base rachis to the top of the uppermost spikelet excluding the awn. The FLW measurement (mm) was taken at the widest part of the flag leaf. GW per plot was represented by the mean of three independent 100-grain samples (HGW). KL, KW and KT of 50 kernels randomly selected from each plot were measured using a vernier caliper, and the mean was used for further analysis.

Statistical analysis

Pearson correlation and analysis of variance in general linear model were carried out using the SPSS statistical package (SPSS Inc., Chicago, IL). Fisher's least significant difference (LSD) test was applied for comparison of two samples.

Results

NIL development

In the crosses for development of single QTL NILs, 10–40 plants at each backcross generation were genotyped using markers linked to the target QTLs to identify plants heterozygous at the target QTL intervals (forward selection) (Table 2). As expected, nearly half of the surveyed plants carried the target QTLs regardless of the recurrent parents and QTL intervals. In the cross for developing NIL containing *QGw.nau-4B* and *QGw.nau-5A,* 10–20 plants were genotyped using the linked markers at each backcross generation and 2–4 plants heterozygous at the target QTL intervals were identified.

To speed up the recovery of genetic composition of the backcross parents in the offspring, BS was performed starting at BC_2F_1. According to the marker genotypes of WSB, NW70, NW23, and NW53 (Xue et al. 2008), 69, 58 and 75 markers, distributed over all the chromosome arms, were polymorphic between the crossed parents and could be used for background genotyping. At BC_2F_1, the plants with the target QTLs were genotyped with 10–13 of these markers. Plants with as high as 90.9 and 100 % RGC were obtained for further backcross in the NW70 and NW23 crosses. In the NW53 cross, only two plants carried both *QGw.nau-4B* and *QGw.nau-5A* and one of them had as high as 76.9 % RGC. At BC_3F_1, another 15–19 markers were used in the background genotyping of plants with the

Table 2 Generation to generation selection for lines with the targeted QTL intervals and the highest recurrent parent genetic composition (RGC %) in the WSB background

Generation	*QGw.nau-2D* (NW70)				*QGw.nau-5A* (NW23)				*QGw.nau-4B&5A* (NW53)			
	No. plants screened	No. plants selected	No. markers for BS	Highest RGC %	No. plants screened	No. plants selected	No. markers for BS	Highest RGC %	No. plants screened	No. plants selected	No. markers for BS	Highest RGC %
BC_1F_1	10	3	–	–	10	8	–	–	10	4	–	–
BC_2F_1	20	8	11	90.9	20	10	10	100	20	2	13	76.9
BC_3F_1	40	19	15	96.2	40	13	15	100	19	3	19	84.4
BC_3F_2	54	14	43	98.6	78	17	33	100	54	2	43	93.3

The donor parents were indicated in the parentheses

BS background selection

Table 3 Hundred-grain weight of the QTL NILs and recurrent parents

Variety/line	Jiangpu		Fengyang	
	2013	2014	2013	2014
WSB	3.4 ± 0.01	4.5 ± 0.02	3.5 ± 0.03	4.6 ± 0.02
NIL-*QGw.nau-2D* (WSB)	3.6 ± 0.02**	4.8 ± 0.03**	3.8 ± 0.02**	4.8 ± 0.02**
NIL-*QGw.nau-4B* (WSB)	3.9 ± 0.03**	4.9 ± 0.03**	3.9 ± 0.02**	5.1 ± 0.02**
NIL-*QGw.nau-5A* (WSB)	3.7 ± 0.02**	4.9 ± 0.02**	3.8 ± 0.02**	5.0 ± 0.03**
NIL-*QGw.nau-4B&5A* (WSB)	4.00 ± 0.01**	5.2 ± 0.04**	4.1 ± 0.04**	5.2 ± 0.03**
W6	5.0 ± 0.03	5.0 ± 0.04	4.7 ± 0.00	5.3 ± 0.02
NIL-*QGw.nau-4B* (W6)	5.4 ± 0.04**	5.5 ± 0.04**	5.2 ± 0.02**	5.8 ± 0.02**

Data are given as the mean ± SD

** Indicate significant difference from WSB (Wangshuibai) or W6 (Wenmai 6) at $p = 0.01$

target QTLs. Plants with as high as 96.2, 100, and 84.4 % RGC were selfed to generate BC_3F_2 populations. At BC_3F_2, plants homozygous at the target QTL intervals were first identified using the linked markers in the three populations and then genotyped with polymorphic markers which had not been used in the background genotyping in the previous backcross generations. The plants with the highest RGC were retained as NILs (Table 2). In the NW53 cross, plants with single QTLs and with both *QGw.nau-4B* and *QGw.nau-5A* were obtained; however, due to the small population size and lower level of background recovery, plants with homozygous *QGw.nau-5A* and higher than 90 % RGC were not obtained.

A similar scheme was applied to development of *QGw.nau-4B* NIL with the W6 background, but the forward selection was not performed at BC_1F_1. Nine plants heterozygous at the *QGw.nau-4B* interval were identified from 15 BC_2F_1 plants. After survey of the parents with markers mapped to the wheat genome, 81 markers polymorphic between the parents, distributed over all the 42 chromosome arms, were identified for BS. The BC_2F_1 plants with the target QTL were genotyped with 26 of these polymorphic markers. One plant with 88.5 % RGC (the highest) was selfed. Of 138 BC_2F_2 plants, 27 were homozygous at the *QGw.nau-4B* interval. After examined with the remaining 55 polymorphic markers, one plant with the highest RGC (95 %) was retained as NIL.

GW evaluation

To assess the effects of each QTL on GW and other agronomical traits, the NILs and their recurrent parents were evaluated in two-year two-location field trials. Analysis of variance indicated significant between-genotype and between-year variations of GW (data not shown). However, the between-location variation was not significant; this was expected since the two locations are in the same latitude and only about 200 km away from each other.

The QTL NILs consistently produced heavier kernels than the respective recurrent parents independent of the locations, years and background genotypes (Table 3). A similar result was obtained in 2011 season when the WSB background materials were evaluated in a field house (data not shown). Since the WSB background materials suffered from serious lodging during the mature stage in 2013, the GW was lower in the 2012–2013 trial than in the

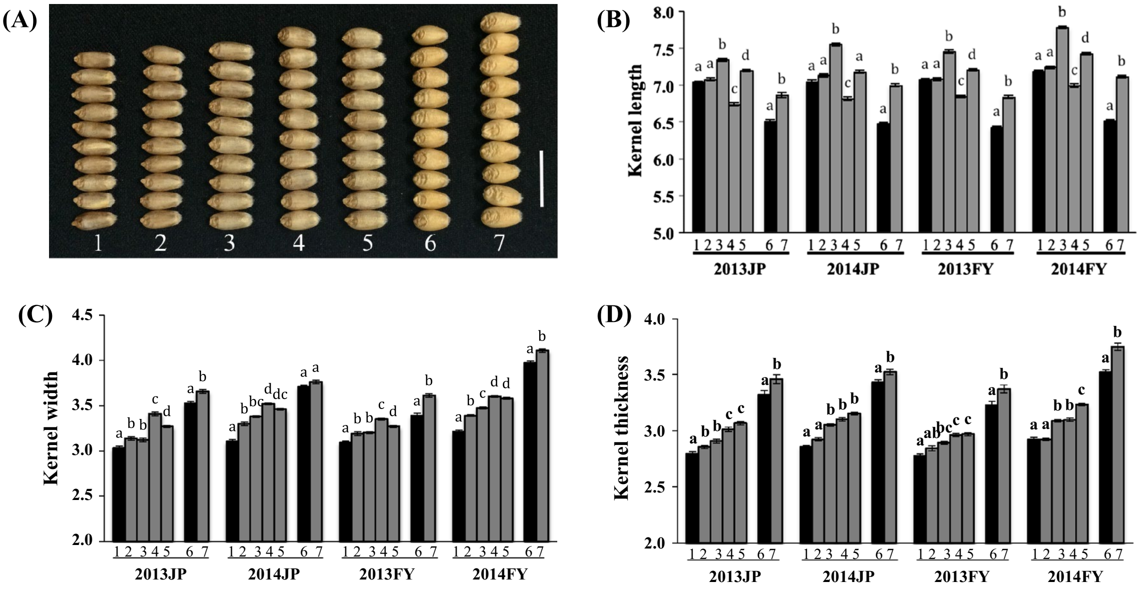

Fig. 1 KSS of NILs and the recurrent parents. **A** Visual view of KSS: *Scale Bar* 1 cm. **B–D** KL, KW, KT in the four trials. *Data* were shown as the mean ± SD. *Different letters* indicate significantly different at $p = 0.05$. *1* Wangshuibai, *2* NIL-*QGw.nau-2D*, *3* NIL-*QGw.nau-4B*, *4* NIL-*QGw.nau-5A*, *5* NIL-*QGw.nau-4B&5A*, *6* Wenmai 6, *7* NIL-*QGw.nau-4B* (Wenmai 6)

2013–2014 trial. Nevertheless, the HGW of the QTL NILs was still significantly higher than that of WSB in 2013.

Effects of the individual QTLs on GW

QGw.nau-4B showed the largest effect on GW; its presence raised HGW by 0.4–0.5 g with an average of 0.45 g. *QGw.nau-4B* showed even greater effect in W6 background and increased HGW by 0.48 g on the average. The effect of *QGw.nau-5A* was second to *QGw.nau-4B*; it conditioned an average of 0.35 g HGW increase in the four trials. The HGW difference between the *QGw.nau-2D* NIL and WSB was 0.2–0.3 g, with an average of 0.25 g. At $p = 0.05$, in at least three of the four trials, *QGw.nau-4B* had significant larger effect than *QGw.nau-5A,* and the latter had significant larger effect than *QGw.nau-2D*.

The effect of pyramiding of *QGw.nau-4B* and *QGw.nau-5A* on GW was evident. The line with both QTLs produced 0.6–0.7 g heavier HGW than WSB and the HGW difference was also significant at $p = 0.05$ when compared with the NILs with either *QGw.nau-4B* or *QGw.nau-5A*, implying the two QTLs functioned mainly additively in determining GW.

Effects of the individual QTLs on kernel dimensions

The kernel dimensions of the NILs and their recurrent parents were investigated. The kernel shape was visually different among the lines (Fig. 1A). Interestingly, the three GW QTL intervals appeared to have different effects on KL, KW and KT. The kernels of *QGw.nau-4B* NILs were significantly longer, wider, and thicker than the kernels of both WSB and W6 (Fig. 1B, C). Particularly, the *QGw.nau-4B* NIL had the longest kernels among the three WSB background NILs with a single GW QTL. Similar to the *QGw.nau-4B* interval, the *QGw.nau-5A* interval conditioned wider and thicker kernels. The *QGw.nau-5A* interval had the largest effect on kernel width; however, its introduction into WSB resulted in significantly shorter kernels. Pyramiding of the *QGw.nau-4B* and *QGw.nau-5A* intervals improved all three dimensions of the kernels (Fig. 1B, C), as a consequence, increased the GW to even higher level than the individual QTLs. Compared with WSB*, QGw.nau-2D* NIL had significantly wider kernels ($p = 0.05$) and slightly longer and thicker seeds, but the differences in KL and KT were not statistically significant.

Agronomic traits of the NILs

Six agronomical traits, including TN, PH, SPL, SPN, FLW and FLL, were surveyed in the four trials. No significant difference was found between NILs and the respective recurrent parents for TN, SPN, and FLL. However, the *QGw.nau-4B* NIL in W6 background was taller, the *QGw.nau-2D* NIL had longer spikes, and the *QGw.nau-5A* NIL, as well as the *QGw.nau-4B&5A* line, had wider flag leaves (Table 4).

Discussion

For breeders to use at ease in their breeding programs the QTL mapping information obtained with primary mapping

Table 4 Comparison of traits for particular NILs that showed significant difference from the recurrent parents

Variety/line	Trait	2013 Jiangpu	2014 Jiangpu	2013 Fengyang	2014 Fengyang
WSB	SPL	12.6 ± 0.25	12.9 ± 0.13	13.5 ± 0.20	12.7 ± 0.15
NIL-*QGw.nau-2D*		13.6 ± 0.34*	13.8 ± 0.15**	14.3 ± 0.22*	13.4 ± 0.17**
WSB	FLW	1.6 ± 0.03	1.5 ± 0.01	1.5 ± 0.02	1.5 ± 0.02
NIL-*QGw.nau-5A*		1.9 ± 0.05**	1.7 ± 0.02**	1.7 ± 0.03*	1.7 ± 0.03**
NIL-*QGw.nau-4B&5A*		1.7 ± 0.03**	1.7 ± 0.02**	1.7 ± 0.03*	1.7 ± 0.01**
W6	PH	62.7 ± 0.86	63.1 ± 0.50	65.1 ± 0.40	63.1 ± 0.54
NIL-*QGw.nau-4B* (W6)		73.8 ± 1.42**	79.6 ± 1.48**	81.1 ± 1.25**	74.8 ± 1.79**

Data are given as the mean ± SD

SPL spike length, *FLW* flag leaf length, *PH* plant height, *WSB* Wangshuibai, *W6* Wenmai 6

*, ** indicate significant at $p = 0.05$ and 0.01, respectively

populations, QTLs validation in different populations or through NIL evaluation is a must. As part of our efforts to determine the genetic factors contributing to the superior performance of elite cultivar Nanda2419 or Mentana in breeding programs and field production, the effects of three QTLs assigned to Nanda2419 that affected GW, including *QGw.nau-2D*, *QGw.nau-4B* and *QGw.nau-5A*, were examined through NIL evaluation in this study. Introduction of any one of these QTLs significantly increased GW in the recurrent parents and pyramiding of *QGw.nau-4B* and *QGw.nau-5A* exhibited additive effects. It was concluded that the three GW QTLs from Nanda2419 are useful for wheat yield breeding.

The effects of *QGw.nau-4B* and *QGw.nau-5A* intervals on GW have also been documented in other mapping including association-mapping studies (Breseghello and Sorrells 2006; Cuthbert et al. 2008; Gegas et al. 2010; Sun et al. 2010; Zheng et al. 2010; Mir et al. 2012; Wang et al. 2012a; Tyagi et al. 2014). Both QTL intervals showed larger effects relative to *QGw.nau-2D*. When *QGw.nau-4B* was introduced into the improved cultivar W6, its effect was even greater and reached 9.5 %, which would be equivalent to the yield increase level, assuming the kernel number per spike and spike numbers per unit area are not changed. In the WSB NIL containing *QGw.nau-4B* and *QGw.nau-5A*, GW increased by 14 %, ranging from 9.9 to 16.7 %. *QGw.nau-2D* was not detected in the RIL population derived from Nanda2419 × WSB due to the use of a relatively high QTL declaration threshold (Jia et al. 2013) but was detected by comparing a NIL of this interval with its recurrent parent ‘Mianyang 99-323’ (Wu et al. 2014). The corresponding interval was also linked to GW in other mapping studies (Groos et al. 2003; Huang et al. 2006; Su et al. 2009).

KSS is the major GW determinant and affected by KL, KW, KT, and filling degree. Therefore, it was not surprising that the GW increase caused by the three QTLs was directly related to the kernel dimension change. This type of association has been demonstrated in cereal crops in many studies (Dholakia et al. 2003; Sun et al. 2009; Gegas et al. 2010; Ramya et al. 2010; Cui et al. 2014). Similar to rice *GS3* (Fan et al. 2006), the *QGw.nau-4B* interval showed smaller effects on width and thickness but larger effect on KL; it increased KL by an average of 6.3–7.3 % depending on genetic backgrounds. Different from the *QGw.nau-4B* interval and rice *GW2* that increases KW and GW and has minor positive effects on KL and KT (Song et al. 2007), the *QGw.nau-5A* interval had major effect on KW and conditioned thicker but shorter kernels, which is much like *GW8* (*OsSPL16*) that increases KW and yield and reduces KL as a subsequence of positive regulation of cell proliferation (Wang et al. 2012b). Sun et al. (2010) and Gegas et al. (2010) reported the association of the same interval with kernel diameter and kernel shape, respectively. The *QGw.nau-2D* interval was only related to KW and GW and its association with KW has been reported in other studies (Dholakia et al. 2003; Breseghello and Sorrells 2006; Huang et al. 2006; Okamoto et al. 2012; Tyagi et al. 2014; Williams and Sorrells 2014).

However, the association of kernel dimension parameters with GW was not always consistent in different studies. In the association-mapping study by Breseghello and Sorrells (2006), the *QGw.nau-5A* corresponding interval showed significant effect on KL instead of on KW. In a DH population derived from Cayuga × Caledonia, the 5A GW QTL interval was associated with KW but not with GW (Williams and Sorrells 2014). The *QGw.nau-4B* corresponding interval, mainly associated with KW in addition to GW in a double haploid population derived from ‘Avalon’ × ‘Cadenza’ (Gegas et al. 2010), was only related to KL in a RIL population derived from ‘Chuan 35050’ × ‘Shannong 483’ (Sun et al. 2009). Therefore, it is still to be determined if the association of GW and KSS factors to common chromosomal intervals is due to pleiotropy or gene linkage. Moreover, in some studies not all the KSS QTL intervals were linked to GW and the vice versa (Sun et al. 2009; Cui et al. 2014; Russo et al. 2014; Williams and Sorrells 2014). These results, on one hand, suggest complexity of the genetic mechanisms underlying GW and KSS; on the other hand, they necessitate verification since QTL allelic variations exist in different germplasms and genetic backgrounds as well as environments all affect GW and KSS, which could lead to different results.

In comparison of six agronomical traits other than the target trait between the NILs and the respective recurrent parents, even though no significant difference was found for half of these traits, *QGw.nau-2D*, *QGw.nau-4B* and *QGw.nau-5A* NILs in certain backgrounds showed longer spikes, taller plants and wider flag leaves, respectively. We found these associations were less likely due to background noise, and more likely resulted from genetic linkage, pleiotropic effect, or even physiological association, as larger spike and wider leaf could provide more photo-assimilates for kernel filling and have been related to yield or GW (Moghaddam et al. 1997; Donmez et al. 2001; Cui et al. 2003, Wang et al. 2011; Jia et al. 2013). The association of chromosomal regions with multiple agronomic QTLs is common in crops. In the Nanda2419 × WSB RIL population, there was one PH QTL with the LOD score peak adjacent to that of *QGw.nau-4B* (Jia et al. 2013), which could explain why the *QGw.nau-4B* NIL in W6 background was taller than W6. Nevertheless, the introduction of *QGw.nau-4B* interval into WSB did not affect plant height, suggesting that the association of the 4B GW QTL interval with plant height is likely due to linkage and could be broken through genetic recombination. The association of the *QGw.nau-2D* interval with spike length was in support of the findings of Wu et al. (2014) in a different genetic background. Further studies are required to find out the causes that determine the association of the *QGw.nau-2D* and *QGw.nau-5A* intervals with traits other than GW.

Using a marker-assisted forward and background selection strategy, we were able to develop QTL NILs with over 90 % background recovery through just 2–3 rounds of backcross, which illustrated the feasibility of marker-assisted breeding for agronomical traits controlled by polygenes even with minor effects. In this study, the population size surveyed in each generation was small, ranging from 10–40 plants, which is easy to handle for breeders. In developing *QGw.nau-4B* NIL in the W6 background, a line with 95 % background recovery was obtained through only 2 rounds of backcross and one generation of selfing. To save the workload, background selection for this NIL development was only conducted at BC_2F_1 and BC_2F_2. Because of the small population size (10–20 plants) used for selection of plants with two QTLs, we failed in obtaining plants with the target QTLs and a background recovery level comparable to that achieved in crosses for single QTL selection due to much fewer plants with both QTLs. It is postulated that using a population size of 30–40 plants in the backcross generations would stand a good chance obtaining plants with ideal background recovery level and also the target QTLs.

With the release of more and more genome resources in wheat, isolation and cloning of genes underlying agronomically important traits will become more feasible in the near future. The NILs developed in this study could be used for construction of secondary segregation populations by crossing with the recurrent parents, in which the target QTLs could segregate like single genes, and recombinants can therefore be easily identified for fine mapping, and thus useful for cloning of the GW-related QTLs and unraveling of kernel development mechanisms. Moreover, since the NILs had combinations of the improved grain weight with FHB resistance of WSB and with the overall merit of W6, they could be employed in breeding programs with high yielding and FHB resistance as the objectives.

Author contribution statement YL Huang conducted NIL development, phenotyping, data analysis and prepared the draft; ZX Kong contributed to field trials and data collection; XY Wu initiated the crosses and selection, RR Cheng and D Yu participated phenotyping; Z Ma conceived the project, designed the experiments and reviewed the article.

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Compliance with ethical standards

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

References

Ammiraju J, Dholakia B, Santra D, Singh H, Lagu M, Tamhankar S, Dhaliwal H, Rao V, Gupta V, Ranjekar P (2001) Identification of inter simple sequence repeat (ISSR) markers associated with seed size in wheat. Theor Appl Genet 102:726–732

Bassam B, Caetano-Anolles G, Gresshoff P (1991) Fast and sensitive silver staining of DNA in polyacrylamide gels. Anal Biochem 196:80–83

Blair CP (1992) Factor regression for interpreting genotype environment interaction in bread wheat trial. Theor Appl Genet 83:1022–1026

Botwright TL, Condon AG, Rebetzke GJ, Richards RA (2002) Field evaluation of early vigour for genetic improvement of grain yield in wheat. Aust J Agric Res 53:1137–1145

Breseghello F, Sorrells ME (2006) Association mapping of kernel size and milling quality in wheat cultivars (*Triticum aestivum* L.). Genetics 172:1165–1177

Breseghello F, Sorrells ME (2007) QTL analysis of kernel size and shape in two hexaploid wheat mapping populations. Field Crop Res 101:172–179

Calderini D, Dreccer M, Slafer G (1995) Genetic improvement in wheat yield and associated traits. A re-examination of previous results and the latest trends. Plant Breed 114:108–112

Campbell KG, Bergmem CJ, Gualberto DG, Anderson JA, Giroux MJ, Hareland G et al (1999) Quantitative trait loci associated with kernel traits in a soft × hard wheat cross. Crop Sci 39:1184–1195

Chastain TG, Ward KJ, Wysocki DJ (1995) Stand establishment responses of soft white winter wheat to seedbed residue and seed size. Crop Sci 35:213–218

Cui K, Peng S, Xing Y, Yu S, Xu C, Zhang Q (2003) Molecular dissection of the genetic relationships of source, sink and transport tissue with yield traits in rice. Theor Appl Genet 106:649–658

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

Cuthbert J, Somers D, Brule-Babel A, Brown P, Crow G (2008) Molecular mapping of quantitative trait loci for yield and yield components in spring wheat (*Triticum aestivum* L.). Theor Appl Genet 117:595–608

Deng S, Wu X, Wu Y, Zhou R, Wang H, Jia J, Liu S (2011) Characterization and precise mapping of a QTL increasing spike number with pleiotropic effects in wheat. Theor Appl Genet 122:281–289

Dholakia BB, Ammiraju JSS, Singh H, Lagu MD, Röder MS, Rao VS et al (2003) Molecular marker analysis of kernel size and shape in bread wheat. Plant Breed 122:392–395

Donmez E, Sears RG, Shroyer JP, Paulsen GM (2001) Genetic gain in yield attributes of winter wheat in the Great Plains. Crop Sci 41:1412–1419

Fan C, Xing Y, Mao H, Lu T, Han B, Xu C, Li X, Zhang Q (2006) GS3, a major QTL for grain length and weight and minor QTL for grain width and thickness in rice, encodes a putative transmembrane protein. Theor Appl Genet 112:1164–1171

Gegas VC, Nazari A, Griffiths S, Simmonds J, Fish L, Orford S, Sayers L, Doonan JH, Snape JW (2010) A genetic framework for grain size and shape variation in wheat. Plant Cell 22:1046–1056

Groos C, Robert N, Bervas E, Charmet G (2003) Genetic analysis of grain protein-content, grain yield and thousand-kernel weight in bread wheat. Theor Appl Genet 106:1032–1040

Gupta P, Mir R, Mohan A, Kumar J (2008) Wheat genomics: present status and future prospects. Int J Plant Genom. doi:10.1155/2008/896451

Huang X, Coster H, Ganal M, Roder M (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

Huang X, Kempf H, Ganal M, Roder M (2004) Advanced backcross QTL analysis in progenies derived from a cross between a German elite winter wheat variety and a synthetic wheat (*Triticum aestivum* L.). Theor Appl Genet 109:933–943

Huang X, Cloutier S, Lycar L, Radovanovic N, Humphreys D, Noll J, Somers D, Brown P (2006) Molecular detection of QTLs for agronomic and quality traits in a doubled haploid population derived from two Canadian wheats (*Triticum aestivum* L.). Theor Appl Genet 113:753–766

Jia H, Wan H, Yang S, Zhang Z, Kong Z, Xue S, Zhang L, Ma Z (2013) Genetic dissection of yield-related traits in a recombinant inbred line population created using a key breeding parent in China's wheat breeding. Theor Appl Genet 126:2123–2139

Li Y, Fan C, Xing Y, Jiang Y, Luo L, Sun L, Shao D, Xu C, Li X, Xiao J (2011) Natural variation in *GS5* plays an important role in regulating grain size and yield in rice. Nat Genet 43:1266–1269

Ma ZQ, Sorrells ME (1995) Genetic analysis of fertility restoration in wheat using restriction fragment length polymorphisms. Crop Sci 35:1137–1143

Maphosa L, Langridge P, Taylor H, Parent B, Emebiri L, Kuchel H, Reynolds M, Chalmers K, Okada A, Edwards J, Mather D (2014) Genetic control of grain yield and grain physical characteristics in a bread wheat population grown under a range of environmental conditions. Theor Appl Genet 127:1607–1624

Mir RR, Kumar N, Jaiswal V, Girdharwal N, Prasad M, Balyan HS, Gupta PK (2012) Genetic dissection of grain weight in bread wheat through quantitative trait locus interval and association mapping. Mol Breed 29:963–972

Moghaddam M, Ehdaie B, Waines JG (1997) Genetic variation and interrelationships of agronomic characters in landraces of bread wheat from southeastern Iran. Euphytica 95:361–369

Neumann K, Kobiljsdki B, Denčić S, Varshney RK, Börner A (2011) Genome-association mapping-a case study in bread wheat (*Triticum aestivum* L.). Mol Breed 27:37–58

Okamoto Y, Kajimura T, Ikeda TM, Takumi S (2012) Evidence from principal component analysis for improvement of grain shape-and spikelet morphology-related traits after hexaploid wheat speciation. Genes Genet Syst 87:299–310

Quarrie S, Steed A, Calestani C, Semikhodskii A, Lebreton C, Chinoy C, Steele N, Pljevljakusić D, Waterman E, Weyen J, Schondelmaier J, Habash D, Farmer P, Saker L, Clarkson D, Abugalieva A, Yessimbekova M, Turuspekov Y, Abugalieva S, Tuberosa R, Sanguineti M, Hollington P, Aragués 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

Ramya P, Chaubal A, Kulkarni K, Gupta L, Kadoo N, Dhaliwal HS, Chhuneja P, Lagu M, Gupta V (2010) QTL mapping of 1000-kernel weight, kernel length, and kernel width in bread wheat (*Triticum aestivum* L.). J Appl Genet 51:421–429

Reif JC, Gowda M, Maurer HP, Longin CFH, Korzun V, Eb-meyer E, Bothe R, Pietsch C, Würschum T (2010) Association mapping for quality traits in soft winter wheat. Theor Appl Genet 122:961–970

Röder M, Huang X, Börner A (2008) Fine mapping of the region on wheat chromosome 7D controlling grain weight. Funct Integr Genomics 8:79–86

Russo M, Ficco D, Laidò G, Marone D, Papa R, Blanco A, Gadaleta A, De Vita P, Mastrangelo A (2014) A dense durum wheat × *T. dicoccum* linkage map based on SNP markers for the study of seed morphology. Mol Breed 34:1579–1597

Rustgi S, Shafqat MN, Kumar N, Baenziger PS, Ali ML, Dweikat I, Campbell BT, Gill KS (2013) Genetic dissection of yield and its component traits using high-density composite map of wheat chromosome 3A: bridging gaps between QTLs and underlying genes. PLoS One 8:e70526

Shomura A, Izawa T, Ebana K, Ebitani T, Kanegae H, Konishi S, Yano M (2008) Deletion in a gene associated with grain size increased yields during rice domestication. Nat Genet 40:1023–1028

Simmonds J, Scott P, Leverington-Waite M, Turner A, 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

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

Su J, Zheng Q, Li H, Li B, Jing R, Tong Y, Li Z (2009) Detection of QTLs for phosphorus use efficiency in relation to agronomic performance of wheat grown under phosphorus sufficient and limited conditions. Plant Sci 176:824–836

Su Z, Hao C, Wang L, Dong Y, Zhang X (2011) Identification and development of a functional marker of *TaGW2* associated with grain weight in bread wheat (*Triticum aestivum L.*). Theor Appl Genet 122:211–223

Sun X, Wu K, Zhao Y, Kong FM, Han GZ, Jiang HM, Huang XJ, Li RJ, Wang HG, Li SS (2009) QTL analysis of kernel shape and weight using recombinant inbred lines in wheat. Euphytica 165:615–624

Sun X, Marza F, Ma H, Carver B, Bai G (2010) Mapping quantitative trait loci for quality factors in an inter class cross of US and Chinese wheat. Theor Appl Genet 120:1041–1051

Tsilo TJ, Hareland GA, Simsek S, Chao S, Anderson JA (2010) Genome mapping of kernel characteristics in hard red spring wheat breeding lines. Theor Appl Genet 121:717–730

Tyagi S, Mir RR, Balyan HS, Gupta PK (2014) Interval mapping and meta-QTL analysis of grain traits in common wheat (*Triticum aestivum* L.). Euphytica 201:367–380

Underdahl J, Mergoum M, Ransom J, Schatz B (2008) Agronomic traits improvement and associations in hard red spring wheat cultivars released in North Dakota from 1968 to 2006. Crop Sci 48:158–166

Wan X, Weng J, Zhai H, Wang J, Lei C, Liu X, Guo T, Jiang L, Su N, Wan J (2008) Quantitative trait loci (QTL) analysis for rice grain width and fine mapping of an identified QTL allele *gw-5* in a recombination hotspot region on chromosome 5. Genetics 179:2239–2252

Wang J, Liao X, Li Y, Zhou R, Yang X, Gao L, Jia J (2010) Fine mapping a domestication-related QTL for spike-related traits in a synthetic wheat. Genome 53:798–804

Wang P, Zhou GL, Yu HH, Yu SB (2011) Fine mapping a major QTL for flag leaf size and yield-related traits in rice. Theor Appl Genet 123:1319–1330

Wang L, Ge H, Hao C, Dong Y, Zhang X (2012a) Identifying loci influencing 1000-Kernel weight in wheat by microsatellite screening for evidence of selection during breeding. PLoS One 7(2):e29432

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

Williams K, Sorrells ME (2014) Three-dimensional seed size and shape QTL in hexaploid Wheat (*Triticum aestivum* L.) Populations. Crop Sci 54:98–110

Wu X, Cheng R, Xue S, Kong Z, Wan H, Li G, Huang Y, Jia H, Jia J, Zhang L, Ma Z (2014) Precise mapping of a quantitative trait locus interval for spike length and grain weight in bread wheat (*Triticum aestivum* L.). Mol Breed 33:129–138

Xiao Y, Qian Z, Wu K, Liu J, Xia X, Ji W, He Z (2012) Genetic gains in grain yield and physiological traits of winter wheat in Shandong province, China, from 1969 to 2006. Crop Sci 52:44–56

Xue S, Zhang Z, Lin F, Kong Z, Cao Y, Li C, Yi H, Mei M, Zhao D, Zhu H, Xu H, Wu J, Tian D, Zhang C, Ma Z (2008) A high-density intervarietal map of the wheat genome enriched with markers derived from expressed sequence tags. Theor Appl Genet 117:181–189

Xue S, Li G, Jia H, Lin F, Cao Y, Xu F, Tang M, Wang Y, Wu X, Zhang Z, Zhang L, Kong Z, Ma Z (2010) Marker-assisted development and evaluation of near-isogenic lines for scab resistance QTLs of wheat. Mol Breed 15:397–405

Xue S, Xu F, Li G, Zhou Y, Lin M, Gao Z, Su X, Xu X, Jiang G, Zhang S, Jia H, Kong Z, Zhang L, Ma Z (2013) Fine mapping *TaFLW1*, a major QTL controlling flag leaf width in bread wheat (*Triticum aestivum* L.). Theor Appl Genet 126:1941–1949

Zhang W, Chao S, Manthey F, Chicaiza O, Brevis J, Echenique V, Dubcovsky J (2008) QTL analysis of pasta quality using a composite microsatellite and SNP map of durum wheat. Theor Appl Genet 117:1361–1377

Zheng B, Le Gouis J, Leflon M, Rong W, Laperche A, Brancourt-Hulmel M (2010) Using probe genotypes to dissect QTL × environment interactions for grain yield components in winter wheat. Theor Appl Genet 121:1501–1517

Zhou Y, Zhu H, Cai S, He Z, Zhang X, Xia X, Zhang G (2007) Genetic improvement of grain yield and associated traits in the southern China winter wheat region: 1949 to 2000. Euphytica 157:465–473