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Single nucleotide polymorphism tightly linked to a major QTL on chromosome 7A for both kernel length and kernel weight in wheat

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Abstract Thousand-kernel weight (TKW) is one of the major components of grain yield in wheat (*Triticum aestivum*). Identifying major quantitative trait loci (QTLs) for TKW and developing effective markers are prerequisite for success in marker-assisted selection (MAS) to improve wheat yield through breeding. This study mapped a major QTL, designated as *TaTKW-7AL*, for increasing TKW on the long arm of chromosome 7A of 'Clark' to a 1.3-cM interval between single nucleotide polymorphism (SNP) markers *IWB13913* and *IWA5913*. This QTL explained 19.7 % of the phenotypic variation for TKW. A QTL for increasing kernel length (KL), one of the major components of TKW, was mapped in the same interval as *TaTKW-7AL*, suggesting that increased TKW by the QTL in 'Clark' is most likely due to the increased KL. Association analysis on a diversity panel of 200 US winter wheat accessions also identified a haplotype of three SNP markers (IWB13913, IWB6693 and IWA5913) that were tightly associated with the both KL and TKW. The analysis of allele frequencies of the haplotype in the diversity panel suggested that the favorable allele of *TaTKW-7AL* has not been strongly selected for in practice and has potential to be used to improve grain yield in US hard winter wheat breeding. Two user-friendly flanking KASPar markers, IWB13913 and IWA5913, were developed for MAS of *TaTKW-7AL*.

Keywords *Triticum aestivum* · 92K SNP array · *TaTKW-7AL* · KASPar markers · Pleiotropic effect on yield traits

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Introduction

Wheat (*Triticum aestivum* L.) is one of the most important cereal crops worldwide, serving as the staple food for 35 % of the human population (Paux et al. 2008). As world population rises, the global demand for wheat will continuously increase. It is estimated that wheat production need to be increased by 70 % by 2050 to meet the future demands (Mayer et al. 2014). Therefore, developing high-yielding wheat varieties is critical to world food security. Wheat kernel weight, usually measured by thousand-kernel weight (TKW), is one of the three major yield components and has been an important trait to be selected for during domestication and modern breeding to increase wheat grain yield (Gegas et al. 2010). TKW is a complex trait and determined by kernel size (KS) and degree of grain filling in wheat and other cereal crops (Zuo and Li 2014). KS is a highly heritable trait that is determined by kernel length (KL), kernel width (KW) and kernel thickness, whereas the degree of grain filling is more vulnerable to environmental variations (biotic and/or abiotic). Although TKW is controlled by quantitative trait loci (QTLs), it usually has a higher heritability than other yield components and thus becomes a priority trait for improving grain yield in cereal crops (Kumar et al. 2006).

In rice (*Oryza sativa*), TKW and its components have been extensively studied with tremendous progress (Xing and Zhang 2010). Several major QTLs, including *GS3* (Fan et al. 2006; Mao et al. 2010; Takano-Kai et al. 2009), *GS5* (Li et al. 2011), *GS6* (Sun et al. 2013), *qGL3/qGL3.1* (Hu et al. 2012; Qi et al. 2012; Zhang et al. 2012) and *TGW6* (Ishimaru et al. 2013) for KL and TKW, *GW2* (Song et al. 2007) and *GW5/qGW5* (Shomura et al. 2008; Weng et al. 2008) for KW and TKW, and *GIF1* (Wang et al. 2008) for grain filling and TKW, have been genetically characterized, which provides an insight into possible genetic mechanisms that regulate TKW in cereal crops. Compared to rice, research on wheat TKW has lagged behind mainly due to its genome complexity (allohexaploid, $2n = 6X = 42$) and lack of complete reference genome sequence (Paux et al. 2008). To date, only two genes for wheat kernel weight, *TaGW2-6A* and *Ta-GASR7-A1*, have been isolated by homology cloning. *TaGW2-6A* is located on the short arm of chromosome 6A, and it mainly controls the TKW through KW (Su et al. 2011; Yang et al. 2012), whereas *TaGASR7-A1* was mapped on the long arm of chromosome 7A and regulated TKW through KL (Dong et al. 2014, Zhang et al. 2015).

QTL mapping provides a useful tool for dissecting genetic components that control TKW in wheat. To date, QTLs for wheat TKW and its components have been reported in all 21 wheat chromosomes (Okamoto et al. 2013; Rasheed et al. 2014). However, very few markers are available for marker-assisted selection (MAS) because most QTLs only had minor effects and their linked markers are not close enough for prediction of the target QTLs. Therefore, identifying QTLs with major and stable effects on TKW and their tightly linked markers through fine mapping and further validating them in different genetic backgrounds are very important for successes in marker-assisted transfer of these QTLs into elite breeding lines and in map-based cloning of the target genes underlying the QTLs.

In a previous study, a major QTL for TKW was located to an interval of ~13.0 cM using a recombinant inbred line (RIL) population derived from 'Ning7840' × 'Clark' (Sun et al. 2010). Later it was further defined to a smaller interval (~9.0 cM) using the same population (Li et al. 2015). However, the markers did not adequately meet the need for MAS because they are still far from the QTL and are not user-friendly. Also, those markers have not been validated in diverse genetic backgrounds. The objectives of the present study were to (1) identify tightly linked markers to this QTL by increasing the marker density in the QTL region using single nucleotide polymorphism (SNPs) from wheat 90K iSelect assays; (2) dissect its major components that contribute to increased TKW at this QTL; (3) validate the QTL effect in the RIL population under different environments and in a diversity panel of US hard winter wheat; and (4) develop user-friendly markers for MAS in breeding.

Materials and methods

Plant materials and SNP genotyping

The mapping population of 127 RILs was derived from 'Ning7840' × 'Clark' by single-seed descent. 'Ning7840' ('Aurora'/'Anhui 11'/'Sumai3') is a hard red facultative wheat line from China with relatively

low TKW, whereas 'Clark' is a soft red winter wheat cultivar from Purdue University, IN, USA, with relatively high TKW. The diversity panel consists of 135 hard winter wheat accessions and 65 soft winter wheat accessions (Zhang et al. 2010). In the panel, 19 are recently released cultivars, and 181 are elite breeding lines from the 2008 US Southern and Northern Hard Winter Wheat Regional Performance Nursery, the 2008 Hard Winter Wheat Regional Germplasm Observation Nursery, the 2008 Oklahoma State University Elite Yield Trial Nursery, the 2008 US Uniform Eastern Soft Red Winter Wheat Nursery and the 2008 Uniform Southern Soft Red Winter Wheat Nursery (Supplement Table 1).

Evaluation of kernel traits

Both linkage population and the diversity panel were evaluated for kernel traits in both greenhouse and field experiments. The linkage mapping population was phenotyped in the greenhouse at Kansas State University, Manhattan, KS, in 2013 and 2014, and the 2008 field experiments in Enid, OK; the diversity panel was evaluated for kernel traits in the 2012 greenhouse experiment at Kansas State University and in the 2013 field experiments at both Manhattan and Hays, KS. In field, the accessions were arranged in a randomized complete block design with two replications, and each accession was planted in a single row that was 122.0 cm long and 30.0 cm apart. The field management followed local practices without irrigation. In the greenhouse experiments, seedlings of each line were transplanted into a 13-cm^2 plastic pot containing Metro-Mix 360 growing mix (Hummert Int., Earth City, MO) after 7 weeks of vernalization at 6 °C. All entries had two replications (pots) with five plants per replication. Plants were grown in a plastic growing tray containing Metro-Mix 360 growing medium (Hummert Int., Earth City, MO) in the greenhouse with 12 h supplemental daylight with high-pressure sodium lights at 22 ± 5 °C during the day and 17 ± 3 °C during the night. The plants were harvested and hand-threshed after maturity; the grain was dried to constant moisture content (14 %); 300 kernels from each accession were counted and weighed to estimate TKW; and 20 of them were randomly selected to measure KL and KW by lining them up in lengthwise and then widthwise, respectively, along a ruler.

DNA extraction and marker analysis

Genomic DNA from RILs and the diversity panel was isolated using a modified cetyltrimethylammonium bromide protocol (Bai et al. 1999). SSR genotyping followed Liu et al. (2008). The SSR data were scored using GeneMarker version 1.97 (Soft Genetics LLC, State College, PA). SNP genotyping was performed using Infinium^{TM} iSelect SNP genotyping assays with 90K wheat SNPs (Illumina, San Diego, CA, USA) at the USDA Small Grains Genotyping Laboratory in Fargo, ND.

Linkage map construction and QTL analysis

A linkage map of 'Ning7840' × 'Clark' was constructed using 380 SSR markers together with the polymorphic SNP markers from wheat 90K SNP arrays and using IciMapping 3.3 (http://www.isbreeding.net) with a minimum LOD value of 6.0 and Kosambi mapping function. The SSR markers were used to assign linkage groups to chromosomes based on their positions on a previously published wheat consensus map (Somers et al. 2004). QTL mapping was conducted through inclusive composite interval mapping of additive (ICIM-ADD) module in the IciMapping software. A 1.0 cM step was used in scanning. The significant LOD threshold for each data set was determined by 1000 permutations (Doerge and Churchill 1996).

Association analysis

The population structure of the diversity panel was previously determined using software STRUCTURE 2.2 (Zhang et al. 2010). R program was used to conduct analysis of variance to determine marker–trait association in each subpanel and the whole panel. An *F*-test was used to determine the significance between the groups with contrasting alleles in the panel and the subpanels.

Conversion of SNPs to KASPar markers

The SNPs that were closely linked to the major QTL for TKW were converted to Kompetitive allele specific PCR (KASPar) assays (http://www.lgcgroup.com/kasp). Newly designed KASPar makers were evaluated for polymorphisms between the two parents

before genotyping the mapping population. KASPar assays were performed in a 6-μl reaction volume (3 μl 2× KASP Master Mix, 0.0825 μl KASP primer mix and 3 μl genomic DNA at 25 ng/μl), and data were analyzed in an ABI 7900HT real-time PCR system (Life Technology, Grand Island, NY) following the instruction for KASPar analysis (http://www.lgcgroup.com).

Results

Evaluation of wheat kernel traits

'Clark' consistently had significantly higher values of TKW, KL and KW than those of 'Ning7840' in all environments tested. All the three traits showed continuous variations in the RIL population of 'Ning7840'/'Clark,' indicating segregation patterns of quantitative traits (Fig. 1). Transgressive segregations in both directions for all of the three traits suggest that favorable alleles of these traits are distributed in both parents. Significant positive correlations ($P < 0.01$) between TKW and KL ($r = 0.655$), and between TKW and KW ($r = 0.656$) suggest both KW and KL contributed to TKW. Weak positive correlation ($r = 0.348$) between KL and KW indicates they might be controlled by independent QTLs.

SNPs tightly linked to a QTL for TKW on chromosome 7A

Among 90K SNPs scored in the arrays, 8614 polymorphic SNPs that showed less than 5 % missing data in the RIL population, together with 384 SSR markers and one AFLP maker, *Xgcgt.gtg4* that flanked *QTkw.hwwgr-7AL.1* in the previous map (Sun et al. 2010; Li et al. 2015), were used to construct a linkage map. Based on the chromosome locations of mapped SSR markers, the linkage group that corresponds to chromosome 7AL was identified to contain 31 SNPs, 9 SSRs and 1 AFLP covering 125. 9 cM with an average of 3.1 cM per marker. The SSR marker order in the linkage map agrees with that of the wheat consensus map (Fig. 2a, b) (Somers et al. 2004). In this new map, seven SNPs were added in the interval between the previously reported flanking markers X*gcgt.gtg4* and *Xgwm332*. Therefore, the new map was used for further mapping of the QTL.

To identify the tightly linked markers to *QTkw.hwwgr-7AL* and validate its effect in different environments, composite interval mapping was used to determine its location using the new map. *QTkw.hwwgr-7AL*, here re-designated as *TaTKW-7AL*, was consistently mapped on the distal region of *Xgwm332* in the one field and two greenhouse experiments (Fig. 2c) and explained 9.29–16.59 % of the total phenotypic variation of TKW. Using the mean values of TKW over all experiments, *TaTKW-7AL* was delimited into a 1.33-cM interval between two SNPs *IWB13913* and *IWA5913* and accounted for 19.73 % of phenotypic variation. 'Clark' contributed the positive allele that increased TKW by 1.71 g in comparison with 'Ning7840' (Table 1).

Effects of *TaTKW-7AL* on kernel length and kernel width

Both KL and KW are the major components of TKW. To determine the contributor of this TKW QTL, the new 7AL map was used to map QTLs for both traits. One QTL with a major effect on KL was significant across all three environments evaluated, but the QTL was not significant for KW (Table 1). The QTL for KL was colocated with *TaTKW-7AL* between SNPs *IWB13913* and *IWA5913*, and explained 14.82–17.95 % of the total phenotypic variation for KL. 'Clark' contributed the allele for longer kernel. The RILs with the 'Clark' allele had significantly higher ($P < 0.001$) TKW (37.84 g) and longer KL (5.92 mm) than those with the 'Ning7840' allele (34.30 g for TKW and 5.66 mm for KL) (Fig. 3). These results suggest that *TaTKW-7AL* increases TKW mainly through kernel elongation.

Effects of *TaTKW-7AL* on TKW and KL in the diversity panel

A diversity panel of US winter wheat was analyzed using the wheat 90K SNP assays. Three SNPs, *IWB13913*, *IWB6693* and *IWA5913* that tightly link to *TaTKW-7AL,* co-segregated together in the panel (Supplemental Table 1) and formed a haplotype. About one-third of entries (35 %) in the panel carry the Clark's haplotype, suggesting that many of the US winter wheat lines carry the Clark allele, especially soft winter wheat (50.7 %) when the panel was separated into hard and soft groups based on structure analysis (Zhang et al. 2010).

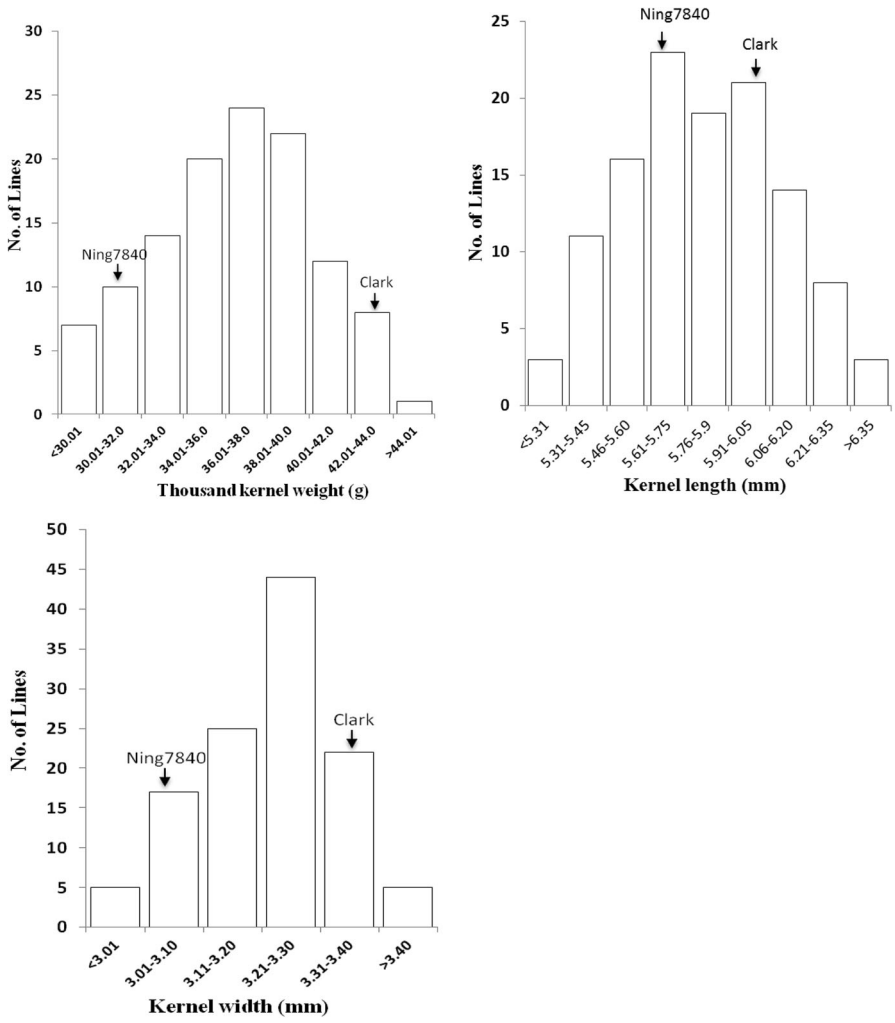

Fig. 1 Frequency distributions for mean of TKW, kernel length and kernel width in the RIL population of 'Ning7840'/'Clark'

In order to verify the effect of *TaTKW-7AL* on kernel traits in the diversity panel, candidate QTL region association analysis was performed using the three tightly linked SNPs (*IWB13913, IWB6693* and *IWA5913*) to *TaTKW-7AL*. In the whole association mapping population, the result showed that marker–trait association was highly significant for KL and TKW ($P < 0.001$). In addition, weak but significant association was also found for KW ($P < 0.05$). The accessions with the 'Clark' haplotype had significant longer and wider kernels, as well as higher TKW, than those with the 'Ning7840' haplotype in the panel. When analysis was done separately for hard and soft subpanels, the differences in KL were still highly significant between the two groups carrying the contrasting haplotypes within each subpanel. The difference was not significant ($P > 0.05$) for KW between the two contrasting groups in either subpanel. For TKW, significant difference was only observed between the two contrasting haplotypes in the soft wheat subpanel ($P < 0.01$), but not in hard wheat subpanel (Table 2). Thus, the Clark allele of *TaTKW-7AL* has played a major role in increasing KL and TKW in soft winter wheat.

User-friendly markers for *TaTKW-7AL*

To develop breeder-friendly markers for MAS of *TaTKW-7AL,* its flanking markers, *IWB13913* and *IWA5913*, were converted into KASPar markers and tested in the RIL population and the diversity panel, respectively (Supplemental Table 2). Analysis of the

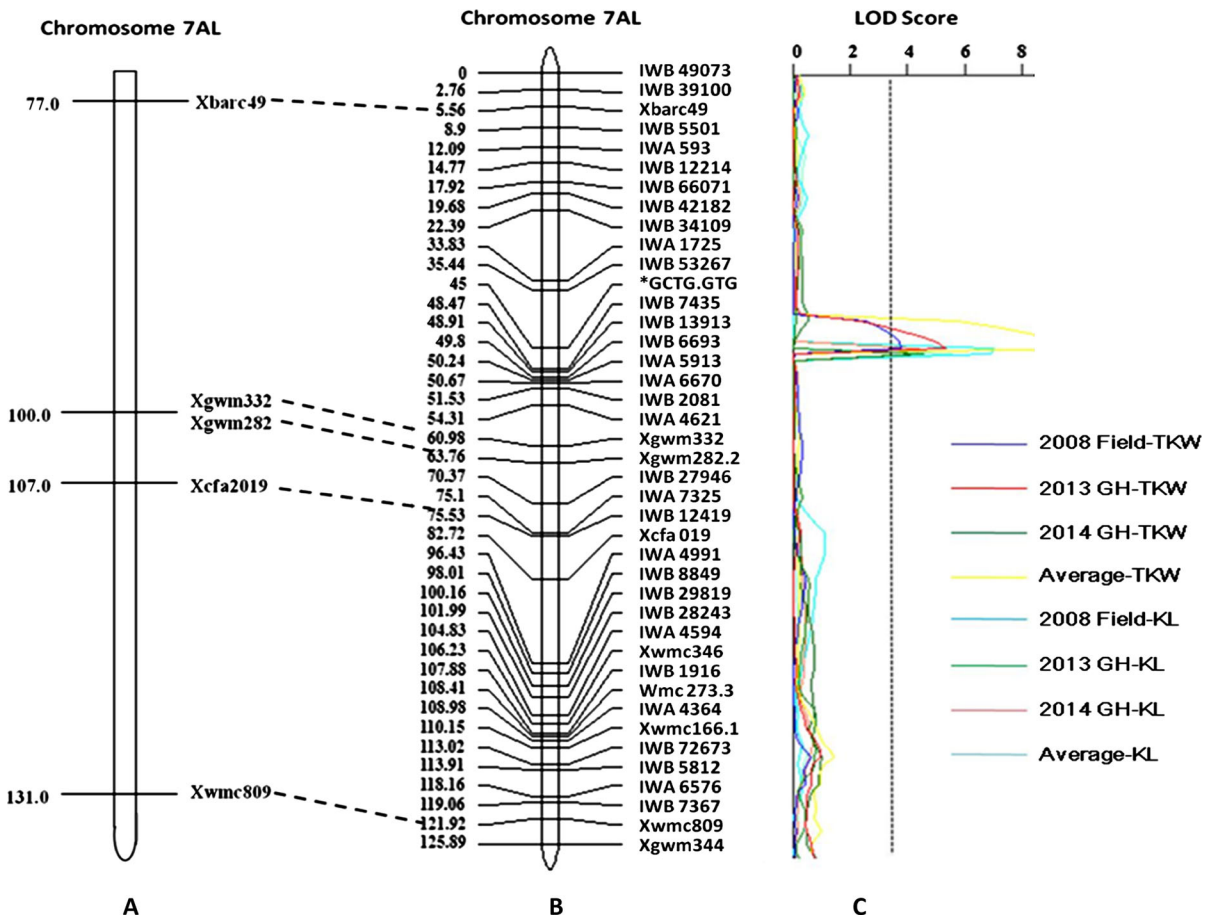

Fig. 2 A high-density linkage map of 7AL and QTL position for thousand-kernel weight (TKW) and kernel length (KL) in the RIL population of 'Ning7840' × 'Clark.' **a** Part of wheat 7AL consensus SSR map (Somers et al. 2004); **b** A 7AL high-density linkage map constructed using wheat 90K SNP arrays and SSR markers; **c** A QTL plot for TKW and KL and KW in different environments in the RIL population of 'Ning7840' × 'Clark.' Field and GH refer to that the phenotype data were collected from field and greenhouse experiments, respectively

two KASPar markers in both the RIL and diversity panel showed that KASPar marker data perfectly matched with the data from the wheat 90K iSelect assays (Supplemental Fig. 1); thus, they are useful for MAS in wheat breeding programs.

Discussion

Several previous studies reported a QTL for TKW near markers *Xgwm332*/*Xgwm282* (~10 cM) on the long arm of chromosome 7A. Groos et al. (2003) reported a QTL for kernel weight in the vicinity of *Xgwm282* and accounted for 5.2–10.3 % phenotypic variation of TKW in six environments. In the population of Chinese Spring × SQ1, a QTL for TKW was colocated with a yield QTL near *Xgwm332*, in which the near-isogenic line carrying the KL allele from SQ1 was about 2.0 g heavier in TKW than the near-isogenic line carrying the contrasting allele (Quarrie et al. 2005, 2006). In the population of MN98550 × MN99394, a QTL that was pleiotropic on TKW and grain morphology was located between markers *XwPt9824* and *XwPt4553* near *Xgwm332,* which explained up to 21 % of the total phenotypic variation for kernel weight, kernel diameter and kernel size in several environments (Tsilo et al. 2010). In a natural population, an SSR marker *Xcfa2257* (8 cM from *Xgwm332* based on the wheat SSR consensus map) showed a significant association with TKW in

Table 1 Summary of QTL data on chromosome 7A for thousand-kernel weight (TKW) and kernel length (KL) in a recombinant inbred line population derived from 'Ning7840' × 'Clark' evaluated in the 2008 field experiment (2008Field) and 2013 (2013GH) and the 2014 (2014GH) greenhouse experiments

Environment/Traits	QTL peak position (cM)	Marker interval	LOD	PVE[a] (%)	Add[b]
2008Field-TKW	50	IWB7435-IWA5913	3.79	11.09	−1.45
2013GH-TKW	49	IWB7435-IWB6693	5.36	16.59	−2.26
2014GH-TKW	50	IWB13913-IWA5913	4.41	9.29	−1.21
Mean TKW	50	IWB13913-IWA5913	9.84	19.73	−1.71
2008Field-KL	49	IWB7435-IWB6693	7.04	17.95	−0.13
2013GH-KL	50	IWB13913-IWA5913	4.57	15.64	−0.16
2014GH-KL	50	IWB7435-IWA59136	4.37	14.82	−0.14
Mean KL	50	IWB13913-IWA5913	5.53	12.31	−0.11

[a] Phenotypic variation explained by the QTL

[b] *Additive effect* The negative value indicates Clark allele increases phenotypic value

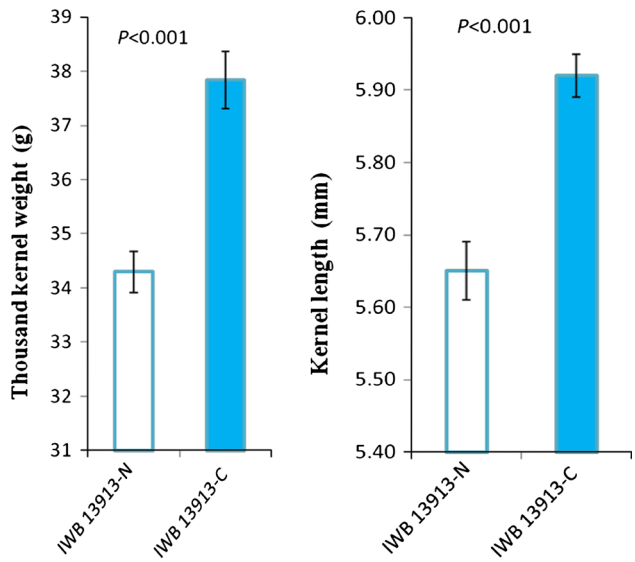

Fig. 3 Mean difference in thousand-kernel weight (TKW) and kernel length (KL) between IWB13913-N and IWB13913-C alleles in the RIL population of 'Ning7840' × 'Clark' (Mean ± SE), where IWB13913-N indicates the 'Ning7840' allele and IWB13913-C indicates the 'Clark' allele

five trials and explained 21.99 % of the total phenotypic variation (Wang et al. 2012a, b). In the current study, a QTL for both TKW and KL was colocated to the vicinity of marker *Xgwm332*. This QTL was significant in multiple environments and showed a major effect on both TKW ($r^2 = 19.73$ %) and KL ($r^2 = 12.31$ %). Thus, it might be the same QTL as those previous reported. Since this major QTL with different names has been reported in various backgrounds, we re-designate it as *TaTKW-7AL*. *TaGASR7-A1* is a QTL for KL in wheat with a pleiotropic effect on TKW and was assigned to chromosome arm 7AL (Dong et al. 2014). Based on the consensus map, Xwmc9, the closest linked marker to *TaGASA7-A1,* is near the centromere (Dong et al. 2014), whereas *TaTKW-7AL* is at least 30 cM distal to Xbarc 49 that is 5 cM distal to Xwmc9 (Somers et al. 2004). Also the marker of *TaGASA7-A1* was not polymorphic between Ning7840 and Clark; therefore, *TaTKW-7AL* is most likely a different QTL from *TaGASA7-A1*.

TKW is mainly determined by kernel size (KS) that can be further dissected into KL and KW and kernel thickness. Thus, large kernel can result from increased

Table 2 Frequency distribution and mean values of a marker haplotype on chromosome 7A associated with thousand-kernel weight (TKW), kernel length (KL) and kernel width (KW) in the whole association panel (WAP) and its two subpanels, hard red winter wheat (HRW) and soft red winter wheat (SRW)

Traits (units)	Population	No. of accession	Frequency of Clark allele (%)	Haplotype		*F* test	*P* value
				Clark (mean ± SE)	Ning7840 (mean ± SE)		
KL (mm)	HRW	135	27.4	5.71 ± 0.036 (mm)	5.57 ± 0.024 (mm)	9.99	0.002**
	SRW	65	50.7	5.77 ± 0.045 (mm)	5.49 ± 0.05 (mm)	17.1	0.00011***
	WAP	200	35.0	5.74 ± 0.029 (mm)	5.55 ± 0.022 (mm)	26.8	0.00000***
KW (mm)	HRW	135	27.4	3.02 ± 0.020 (mm)	3.01 ± 0.010 (mm)	0.21	0.65
	SRW	65	50.7	3.15 ± 0.023 (mm)	3.12 ± 0.019 (mm)	1.13	0.29
	WAP	200	35.0	3.08 ± 0.017 (mm)	3.04 ± 0.010 (mm)	5.95	0.016*
TKW (g)	HRW	135	27.4	34.08 ± 0.43 (g)	33.27 ± 0.29 (g)	2.23	0.14
	SRW	65	50.7	33.92 ± 0.54 (g)	31.82 ± 0.51 (g)	7.88	0.0067**
	WAP	200	35.0	34.00 ± 0.34 (g)	32.91 ± 0.26 (g)	6.41	0.012*

The association analysis was performed using the mean values of the kernel traits in different environments

*, ** and *** indicate significant at 0.05, 0.01 and 0.0001 levels, respectively

kernel length, width or thickness, but they were controlled by independent QTLs (Dholakia et al. 2003; Breseghello and Sorrells 2006; Sun et al. 2009; Rasheed et al. 2014; Wu et al. 2015). In rice, several genes for TKW and KS have been isolated, and the results showed that manipulation of any component of KS could result in the change in TKW. For example, GS3, GS6, qGL3 and TGW6 regulate TKW mainly through modification of KL (Xing and Zhang 2010). However, GW2, GW5/qSW5 and GW8 regulated the TKW mainly through controlling of KW (Zuo and Li 2014). In wheat, *TaGW2-6A* (Su et al. 2011; Yang et al. 2012) and *TaGASR7-A1* (Dong et al. 2014; Zhang et al. 2015), orthologs of rice, were verified to be associated with TKW by regulating the KW and KL, respectively. In the current study, the QTLs for both TKW and KL were co-mapped at the same location on chromosome 7AL, suggesting that *TaTKW-7AL* is pleiotropic on both TKW and KL, and increased TKW by the QTL in 'Clark' is mainly due to elongated kernel length. This result has been validated in the diversity panel of US winter wheat.

The frequency of 'Clark' haplotype (allele) that is associated with the *TaTKW-7AL* for longer and heavier kernels was much higher in soft winter wheat (50.7 %) than in hard winter wheat (27.5 %), suggesting that this QTL has played a more important role in improvement in TKW in US soft winter wheat than in hard winter wheat. Low frequency of the 'Clark' allele in US HWW may partially explain that *TaTKW-7AL* did not show a significant effect on TKW in HWW subpanel and suggests that increase in the 'Clark' allele frequency may facilitate increase in kernel weight in HWW. Because of the major effect of *TaTKW-7AL* on KL and TKW in multiple environments and diverse genetic backgrounds, it might be a good candidate for cloning of a yield-related gene and for studying the mechanisms that regulate wheat TKW.

TKW is one of the major traits that have been gradually altered during domestication and human selection in wheat breeding (Fuller 2007; Brown et al. 2009; Gegas et al. 2010). TKW is a complex trait and controlled by many QTLs including some with minor effects that are easily affected by environmental conditions, which renders difficulties for conventional breeding methods and makes TKW a good candidate trait for MAS. To develop useful markers for MAS, the QTL effects and marker–trait association have to be validated before they can be applied in breeding (Collard and Mackill 2008). Thus, most markers developed from a biparental mapping population may not be useful for MAS in different genetic backgrounds. Although several studies reported QTLs for TKW on 7A in different biparental populations, the QTLs and their linked markers were not validated in a diversity panel. In the present study, the effect of *TaTKW-7AL* was repeatedly evaluated in multiple

environments and marker–trait association was validated in the diversity panel of winter wheat. In the diversity panel, *TaTKW-7AL* showed a significant effect on KL in multiple environments, which confirms that *TaTKW-7AL* is a stable major QTL and its effect on TKW is regulated through KL. Two markers, *IWB13913* and *IWA5913*, flanking *TaTKW-7AL* identified from our linkage mapping co-segregated in the diversity panel and were significantly associated with KL and TKW. Thus, both SNPs can be used to predict *TaTKW-7AL* in breeding programs. *TaTKW-7AL* also showed a significant effect on KW in the diversity panel in multiple environments, whereas in the linkage mapping population, obvious peaks for KW were observed in the QTL region although they were not significant for KW. Therefore, it is possible that *TaTKW-7AL* has a pleiotropic effect on KW.

To successfully deploy *TaTKW-7AL* in wheat breeding programs, user-friendly markers are critical for MAS. KASPar assay is a time saving and cost-effective genotyping assay for uniplex SNP analysis (Semagn et al. 2014; Liu et al. 2014), and it has been widely used in QTL mapping, MAS and fine mapping studies. In the current study, the tightly linked flanking SNPs, *IWB13913* and *IWA5913*, were successfully converted to KASPar assays. These KASPar assays were validated in the diversity panel; thus, they are suitable for MAS in wheat breeding (Supplemental Fig. 1).

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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