Mol Breeding (2015) 35:194
DOI 10.1007/s11032-015-0384-4

Genetic analysis of kernel weight and kernel size in wheat (*Triticum aestivum* L.) using unconditional and conditional QTL mapping

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Received: 23 January 2015 / Accepted: 19 September 2015 / Published online: 3 October 2015

Abstract Thousand kernel weight (TKW) is commonly regarded as a complex trait in wheat. Both unconditional and conditional quantitative trait locus (QTL) analyses were conducted using software QTLNetwork 2.0 in four environments to evaluate the genetic relationships between TKW and kernel size. These analyses involved a set of 182 $F_{8:9}$ recombinant inbred lines derived from "Shannong01-35 × Gaocheng9411" with a genetic linkage map consisting of 503 molecular markers. Additive effects, epistatic effects, and genotype-by-environment (G × E) interactions of QTLs for TKW and kernel size were analyzed. A total of nine additive QTLs for TKW and kernel size were identified using unconditional analysis, which distributed on chromosomes 1B, 1D, 3D, 4B, 5B, and 6A. Among these, four QTLs were detected to be related to several different kernel traits. *QTkw4B.1-7*, *QTkw5B.1-12*, *QTkw5B.1-17*, and *QTkw6A.1-29* were identified through both conditional and unconditional analyses. Seven QTLs for TKW were only identified under conditional QTL mapping. These included conditional QTLs for TKW influenced by or independent of the given kernel size traits. Twelve pairs of epistatic interaction QTLs involving 18 loci for the measured kernel traits were detected in unconditional analysis. The QTLs discovered in the present study through the combination of conditional and unconditional QTL mapping could increase the understanding of the genetic interrelationships between TKW and kernel size traits at the QTL level and provide the guiding information for breeding programs.

Keywords Wheat · Thousand kernel weight · Kernel size · Unconditional QTL analysis · Conditional QTL analysis · Quantitative trait locus

Introduction

Wheat (*Triticum aestivum* L.) is one of the most important food crops around the world. High yield and good quality are the predominant objectives of breeding programs meant to meet the growing demand imposed by the human population and the requirements of food processing. Thousand kernel weight (TKW) is one of the three major yield components, and it contributes more to the increase in yield than either the number of spikes and/or the number of

Electronic supplementary material The online version of this article (doi:10.1007/s11032-015-0384-4) contains supplementary material, which is available to authorized users.

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kernels per spike (Acreche and Slafer 2006). TKW is not only directly related to the grain yield and milling quality of wheat, but also affects seeding vigor and growth indirectly affecting the yield (Botwright et al. 2002; Wiersma et al. 2001). TKW was also found to be closely associated with kernel size traits, such as kernel length (KL), kernel width (KW), kernel thickness (KT), and the kernel length/width ratio (L/W) (Campbell et al. 1999; Dholakia et al. 2003). Kernel size trait usually contributes indirectly to yield by affecting the TKW and can also be considered a predictor of wheat quality associated with milling and processing (Lee et al. 2002; Osborne and Anderssen 2003). TKW and kernel size are typically quantitative traits controlling by multiple genes and impacting by environmental conditions (Ayoub et al. 2002). Recently, a large number of QTL studies have been conducted in an attempt to disclose the genetic basis of TKW and kernel size in wheat. QTLs for TKW have been discovered to be widely distributed on all 21 wheat chromosomes (Araki et al. 1999; Kato et al. 2000; Varshney et al. 2000; Ammiraju et al. 2001; Börner et al. 2002; Groos et al. 2003; Huang et al. 2004, 2006; McCartney et al. 2005; Dilbirligi et al. 2006; Breseghello and Sorrells 2006, 2007; Sun et al. 2009; McIntyre et al. 2010; Ramya et al. 2010). Researchers also found that QTLs for KL are mainly located on chromosomes 1A, 1B, 2B, 2D, 3B, 4A, 4B, 5A, 5B, and 7B (Giura and Saulescu 1996; Campbell et al. 1999; Dholakia et al. 2003; Ramya et al. 2010), and QTLs for KW are mainly on chromosomes 1A, 1B, 2A, 2B, 2D, 3B, 4B, 5A, 5B, 6B, and 7B (Breseghello and Sorrells 2007; Ramya et al. 2010; Sun et al. 2009; Dholakia et al. 2003). QTLs for KT are mainly on chromosomes 1B, 2A, 3B, 4D, 5D, 6A, and 6D (Breseghello and Sorrells 2006; Giura and Saulescu 1996; Tsilo et al. 2010; Sun et al. 2009).

It is found that the QTLs for TKW share a common region or the same QTL with other kernel traits (Peng et al. 2003; Ramya et al. 2010; Sun et al. 2009; Dholakia et al. 2003). However, in these reports, QTL analyses for TKW and kernel size were conducted separately based on their phenotypic values, and moreover, those results explained the genetic associations between kernel traits based solely on whether they were co-located or closely linked. Conventional QTL studies only indicated the correlations for complex traits with the interference of other related traits; they did not evaluate the actual genetic relationships between them. In order to study closely related traits and clarify the contribution of each trait to other related traits at the QTL level, Zhu (1995) proposed the conditional genetics analysis method. This method has been widely used to evaluate genetic effects of quantitative traits at specific developmental stages (Yan et al. 1998; Cao et al. 2001; Wu et al. 2000). Conditional genetic analysis was developed further to study the contributions of single traits to complex trait and the genetic relationships between two traits (Wen and Zhu 2005). In the past several years, conditional QTL mapping has been widely used to investigate genetic basis of complex traits in many crops (Guo et al. 2005; Li et al. 2008; Cui et al. 2013; Zhao et al. 2006). But in wheat, conditional QTL analyses have been mainly conducted on plant height (Wang et al. 2010; Cui et al. 2011). Few conditional QTL analyses have evaluated the genetic relationship between TKW and kernel size traits.

In the present study, both unconditional and conditional QTL analyses were conducted to investigate the underlying genetic basis of TKW and kernel size, and to dissect the genetic relationships between them at the QTL level. The information obtained from the present study could contribute to marker-assisted breeding for kernel traits.

Materials and methods

Plant materials

The recombinant inbred line (RIL) population ($F_{8:9}$) consists of 182 lines derived from a cross of common winter wheat lines Shannong01-35 and Gaocheng 9411. Shannong01-35 featured with the specific large kernel was created by our laboratory and has been widely used as a cross parent to increase kernel weight in breeding program. Gaocheng9411 was from the Gaocheng Institute of Agricultural Sciences in Hebei Province, and this winter wheat line has been widely planted in large areas and used in wheat breeding program for its good bread quality. However, its average yield was comparatively low because of small kernel size and low TKW.

Field trials

The RIL population was grown in Taian (116°36′E, 36°57′N) in 2008, 2009, and 2010 (E1, E2, E3) and in

Suzhou (116°58′E, 33°38′N) in 2010 (E4) as following design. The materials were planted in a randomized complete block with two replications. Every block had three rows with the length of two meter and with a distance of 21 cm. Crop management was performed according to the local cultivation practices. There were no serious damages from plant diseases, insect pests, climate, or lodging during the growing seasons.

Measurement of kernel traits

Kernels from each block were harvested, followed the analysis of kernel traits by using three random samples from each block. TKW was evaluated by weighing 1000 kernels with a precision of 0.01 g. KL, KW, and KT were evaluated using Vernier calipers with a precision of 0.1 mm. Thirty intact kernels of each sample were lined up lengthwise to measure KL, arranged breadthwise and depthwise to measure KW and KT, respectively. The circumference and area of each kernel were measured by seed counting and analysis system Version 1.0 using 100 kernels (Zhejiang Science and Engineering University).

Genotyping and linkage map construction

Total of 503 molecular markers, including 443 DArT markers, 59 SSR markers, and one CAPS marker were used to genotype the RIL population. SSR markers were obtained based on the published sequences of Röder et al. (1998), Pestsova et al. (2000), and GrainGenes 2.0 (http://wheat.pw.usda.gov/GG2/index.shtml and used to investigate polymorphisms between the two parents. The SSR makers of codominant segregation were eventually used to genotyped the RIL population. DArT marker genotyping was performed by Triticarte Pty. Ltd (Australia) as previously described (Akbari et al. 2006).

The genetic linkage groups were constructed using Mapmaker/Exp 3.0 (Lincoln et al. 1992). The linkage map was finally drawn using the software of Mapchart 2.1 (Voorrips 2002), and Kosambi (1943) mapping function was used to convert recombination fractions into cM values as map distances. The 29 linkage groups comprising these 503 markers covered a total length of 4048.5 cM genomic regions with an average interval of 8.13 cM between adjacent markers.

Statistical analysis and QTL mapping

Statistical data analyses were performed using the software SPSS 17.0 (SPSS, Chicago, IL). The differences among the mean values of kernel traits in different environments were estimated by the test of least significant difference (LSD), and the differences were judged statistically significant when $P < 0.01$. Phenotypic correlation coefficients between TKW and kernel size traits were calculated separately for each environment. Analysis of variance (ANOVA) was conducted to test the differences generated by the influence of genotype, environment, and interaction between genotype and environment for each of kernel trait. Broad-sense heritability (h_B^2) for each trait was estimated following

$$h_B^2 = \sigma_g^2 / \left(\sigma_g^2 + \sigma_{ge}^2 / n + \sigma_e^2 / nr \right),$$

where σ_g^2 is the genetic variance, σ_{ge}^2 is the interaction of genotype with environments, σ_e^2 is the environment variance, n is the number of environments, and r is the number of replications per environment (Wyman et al. 1991).

Both conditional and unconditional QTL analyses were performed using QTLNetwork 2.0 (Yang and Zhu 2005; Yang et al. 2007) based on the mixed linear model approach (Wang et al. 1999). Mixed linear model based on composite interval mapping was conducted using forward–backward stepwise with threshold of $P = 0.05$ to select cofactors, and multiple linear regression with 1 cM walking speed and window size set at 10 cM. The significant thresholds for QTL detection were calculated with 1000 permutations and a genomewide error rate of 0.05. A LOD score of 3.0 was used for claiming the presence of QTLs.

QTLs with additive effects and epistatic effects, as well as their environmental interaction effects for the measured kernel traits, were analyzed in unconditional analysis. Conditional analysis was conducted based on the phenotypic values of TKW conditioned on each of TKW component (TKWC). Conditional phenotypic values y(TKW|TKWC) were obtained based on the mixed model approach for the conditional analysis of quantitative traits described by Zhu (1995), where TKW|TKWC indicates TKW conditioned on TKWC (for example, TKW|KL means TKW was conditioned on KL). The software QGA Station 1.0 (http://ibi.zju.

edu.cn/software/qga/) was employed to evaluate the conditional phenotypic values *y*(TKW|TKWC) for conditional analysis. Here, unconditional phenotypic values and conditional phenotypic values obtained from each environment (E1, E2, E3, and E4) and the pooled data collected from the average of the four environments (P) were used for unconditional and conditional QTL analyses.

QTLs were named as followings: Q-abbreviation of the corresponding trait (TKW, KL, KW, KT, L/W) and 1A-7D (the wheat chromosome on which the corresponding QTL was detected), and the last number indicated the marker interval on one chromosome. For example, *QTkw4B-2* indicated that the second interval for TKW was detected on 4B chromosome.

Results

Phenotypic variation and correlation analysis among kernel traits

The mean phenotypic values for kernel traits of the RIL population lines and parents in four environments are presented in Table S1. Shannong01-35 exhibited higher TKW and larger kernel size than that of Gaocheng9411 under all the four environments. Strong transgressing segregations were detected in each kernel trait from all environments, with trait values of RIL progenies being larger or smaller than that of parents, suggesting that alleles with positive effects are distributed among the parents. Every kernel trait segregated continuously in RIL population, with skewness values for all traits were less than 1.0 in absolute value, indicating that these kernel traits followed normal distributions and that phenotypic data in the present study were suitable for QTL analysis. The LSD test demonstrated that non-significant differences in three of four environments for TKW and the mean separation tests for other measured kernel traits could separate the means into 3–4 categories. The results of ANOVA showed that the variances of genotype and environments for all the investigated kernel traits were highly significant at $P < 0.001$ (Table 1). The highly significant differences were also found in genotype-by-environment interactions for all traits except for kernel circumference and kernel area, which were significant at $P < 0.01$. Broad-sense heritability of the investigated kernel traits ranged from 32.8 % (KC) to 71.2 % (TKW), indicating that both genetic and environmental factors played roles in the formation of these measured kernel traits.

All the presented kernel traits were found to be correlated with each other across all of the four

Table 1 Analysis of variance (ANOVA) and the broad-sense heritability (h_B^2) values for the investigated kernel traits in four environments

Trait[a]	Source of variation[b]	MS	*F*	h_B^{2c} (%)
KL	G	9.997	6.167***	69.4
	E	39.824	24.567***	
	G × E	5.628	3.471***	
	Error	1.621		
KW	G	1.470	8.497***	63.1
	E	8.506	49.167***	
	G × E	0.635	3.37***	
	Error	0.173		
KT	G	0.920	11.507***	65.2
	E	4.543	47.323***	
	G × E	0.447	4.465***	
	Error	0.096		
L/W	G	0.725	4.447***	53.4
	E	6.142	37.681***	
	G × E	0.488	2.994***	
	Error	0.163		
TKW	G	464.236	8.861***	71.2
	E	2018.382	38.516***	
	G × E	78.634	1.501***	
	Error	52.393		
KC	G	6.882	1.784***	32.8
	E	156.057	40.446***	
	G × E	4.645	1.204**	
	Error	3.857		
KA	G	6.457	2.440***	47.8
	E	79.606	30.085***	
	G × E	3.325	1.257**	
	Error	2.646		

[a] *KL* kernel length, *KW* kernel width, *KT* kernel thickness, *TKW* thousand kernel weight, *L/W* kernel length/kernel weight ratio, *KC* kernel circumference, *KA* kernel area

[b] *G* Genotype, *E* environment, *G* × *E* genotype-by-environment interaction

[c] h_B^2 broad-sense heritability

** and *** indicate significant level at $P < 0.01$ and $P < 0.001$, respectively

environments (Table S2). Significant positive correlations were observed between TKW and KL, between TKW and KW, between TKW and KT, between TKW and KC, and between TKW and KA, whereas significant negative correlations were observed between TKW and L/W in four environments. The strongest correlation coefficients were detected between TKW and KW, while the lowest between TKW and L/W. The results indicated that KW had the strongest correlation with TKW, followed by KT, KA, KL, and KC.

Additive QTL for kernel traits

Nine unconditional QTLs distributing on chromosomes 1B, 3D, 4B, 5B, 6A, and 6B were identified as shown in Table 2 and Fig. 1. The phenotypic variation explained by individual QTL ranged from 7.61 to 16.85 %. All the identified QTLs except for *QKw3D-11* and *QKl/w4B.1-7* showed positive additive effects, indicating that favorable alleles contributed by Shannong01-35. Four QTLs on chromosomes 1B (*QKl1B.1-47*, *QKL/w1B.1-47*), 5B (*QKw5B.1-12*, *QTkw5B.1-12*), 6A (*QKl6A.1-29*, *QKw6.1-29*, *QKl/w6A.1-29*), and 4B (*QKl4B.1-7*, *QKw4B.1-7*, *QKl/w4B.1-7*, *QTkw4B.1-7*) for different kernel traits were found to share the common marker intervals (Fig. 1).

Four QTLs for KL were identified under all environments, which located on chromosomes 1B, 4B, and 6A, individually explaining 8.21–14.4 % of the phenotypic variation. Among them, *QKl6A.1-29* and *QKl1B.1-18* were the major QTLs detected in more than one environment. *QKl6A.1-29* was detected in Taian 2010 by contributing up to 14.4 % of phenotypic variance. Another major QTL (*QKl1B.1-47*) explained 11.64 % of KL variation.

QKw3D-11, *QKw4B.1-7*, *QKw5B.1-12,* and *QKw6A.1-29* were identified to be associated with KW, which explained 7.61–14.34 % of the phenotypic variation. Notably, *QKw4B.1-7* was the only QTL that was detected in four environments, accounting for 10.45–14.34 % of the phenotypic variance. Of these, *QKw3D-11* was identified with negative additive effects, indicating that favorable alleles were contributed by small kernel parent Gaocheng9411.

For KT, four QTLs were identified on chromosomes 1B, 4B, 6A, and 6B, and explained 8.31–16.85 % of phenotypic variation. Of these, both *QKt1B.1-47* and *QKt4B.1-7* were identified in Taian 2009, accounting for 8.31 and 10.39 % of KT variation, respectively. Another two QTLs, *QKt6B.3-1* and *QKt6A.1-29,* were repeatedly detected in Taian 2010 and environment means. In addition, *QKt6A.1-29* explained the highest phenotypic variation in Taian 2010. All QTLs for KW showed positive additive effects, demonstrating that all favorable alleles were contributed by Shannong01-35.

Four QTLs associated with TKW were detected on chromosomes 4B, 5B, and 6A, explaining 8.43–11.64 % of the phenotypic variation. Among them, *QTkw6A.1-29* was detected across three environments, explaining 8.43–11.64 % of the phenotypic variation. *QTkw4B.1-7* explained 8.61–9.82 % of the phenotypic variation in four environments. The positive additive effects of these QTLs indicated that favorable alleles were contributed by Shannong01-35.

Two L/W QTLs were detected on chromosomes 4B and 1D. *QKl/w4B.1-7* was identified in Taian 2009 and Suzhou 2010, accounting for 12.67 and 15.41 % of phenotypic variation, respectively. *QKl/w1D-12* was detected with negative additive effects and explained 5.51 % of the phenotypic variation.

Epistatic QTLs for kernel traits

Twelve pairs of epistatic effect QTLs were identified for the measured kernel traits, involving 18 loci dispersed on chromosomes 1A, 2A, 3D, 5A, 5B, 5D, 6A, 7A, 7B, and 7D (Table 3). Among these epistatic interactions, none of the loci were significant or detected under additive effects models.

Three pairs of epistatic interactions were identified for KL, involving six loci distributing on chromosomes 2A, 5B, and 7A. Among them, *QKl2A-6/QKl7A-1* and *QKl5B.1-13/QKl5B.2-13* accounted for 10.33 and 10.61 % of KL variation, respectively. Two pairs of epistatic interactions (*QKl2A-6/QKl7A-1* and *QKl2A-7/QKl7A-4*) occurred between loci on chromosomes 2A and 7A. For KW, two pairs of epistatic effect QTLs were identified, including four loci distributing on chromosomes 1A, 2A, and 7B. One pair of epistatic interaction between *QKw1A.1-19* and *QKw1A.1-25* explained 6.58 % of the KW variation.

Three pairs of epistatic interactions were detected for TKW, and all the epistatic interactions occurred between adjacent loci on chromosome 1A. Epistatic interactions between *QTkw1A.1-19* and *QTkw1A.1-25* had the most pronounced effect and accounted for 9.17 % of phenotypic variance. Three pairs of epistatic interactions were detected for L/W, including

Table 2 Additive (A) and additive-by-environment interaction (AE) effects of QTLs for TKW and kernel size

Traits	QTL	Env[a]	Flanking markers	Position	LOD	A[b]	PVE% (A)[c]	PVE% (AE)[d]
KL	***QKl6A.1-29***	E1/E3	*CFE043-TAGW2-CAPS*	132.2	4.77/5.69	0.08/0.04	10.72/14.4	
		ME	*CFE043-TAGW2-CAPS*	131.2	3.45	0.08	3.67	0.53
	QKl1B.1-18	E2/P	*WPT-3753-WPT-1139*	97.5	6.15/5.63	0.10/0.14	10.2/12.88	
	QKl1B.1-47	E4	*WPT-2751-WPT-3465*	111.1	4.68	0.09	11.64	
		ME	*WPT-2751-WPT-3465*	113.1	4.03	0.10	6.8	0.16
	QKl4B.1-7	E1	*WPT-7569-WPT-3908*	99.1	4.28	0.06	8.21	
	QKl1D-4	ME	*WPT-666986-WPT-665480*	3.2	5.32	0.0488	4.4	0.18
	QKl5B.2-16	ME	*WPT-8449-WPT-664746*	102	6.25	0.08	3.03	0.27
KW	***QKw5B.1-12***	E1	*WPT-0103-WPT-4936*	97.8	4.87	0.08	10.8	
		ME	*WPT-0103-WPT-4936*	95.8	4.96	0.04	4.61	0.30
	QKw6A.1-29	E1	*CFE043-TAGW2-CAPS*	131.2	5.06	0.07	10.85	
		ME	*CFE043-TAGW2-CAPS*	129.2	5.36	0.04	5.55	0.23
	QKw4B.1-7	E2/E3/E4/P	*WPT-7569-WPT-3908*	99.1	6.23/4.59/5.87/4.92	0.08/0.05/0.07/0.07	13.96/10.45/12.88/14.34	
		ME	*WPT-7569-WPT-3908*	99.1	6.65	0.04	5.47	0.21
	QKw3D-11	E1	*XGWM456-WPT-668308*	236.2	6.83	−0.05	7.61	
	QKw6B.3-2	ME	*WPT-669607-XGPW1005*	8.4	4.78	0.0381	5.25	0.34
	QKw1B.1-47	ME	*WPT-2751-WPT-3465*	113.1	3.89	0.0493	4.62	0.47
KT	***QKt1B.1-47***	E2	*WPT-2751-WPT-3465*	111.1	5.33	0.05	8.31	
		ME	*WPT-2751-WPT-3465*	113.1	4.01	0.04	2.48	0.27
	QKt4B.1-7	E2	*WPT-7569-WPT-3908*	99.1	5.27	0.06	10.39	
		ME	*WPT-7569-WPT-3908*	99.1	5.67	0.05	4.79	0.47
	QKt6A.1-29	E3/P	*CFE043-TAGW2-CAPS*	131.2	7.68/4.73	0.08/0.058	16.85/11.18	
		ME	*CFE043-TAGW2-CAPS*	131.2	4.64	0.08	4.89	0.46
	QKt6B.3-1	E3/P	*WPT-1325- XGPW1005*	8.4	3.35/4.59	0.05/0.06	8.61/10.21	
	QKt3B.1-20	ME	*WPT-1171-WPT-5906*	215	5.58	0.0231	3.49	0.15
TKW	***QTkw4B.1-7***	E1/E2/E4/P	*WPT-7569-WPT-3908*	99.1	5.02/4.75/4.31/4.66	0.0472/0.05/1.40/1.18	9.82/8.61/8.66/8.72	
		ME	*WPT-7569-WPT-3908*	99.1	5.38	1.72	6.14	0.15
	QTkw5B.1-12	E1	*WPT-0103-WPT-4936*	99.8	5.28	0.97	10.57	
	QTkw5B.1-17	E4	*WPT-666939-WPT-4628*	115.7	5.29	1.35	9.89	
	QTkw6A.1-29	E1/E3/P	*WPT-0832-CFE043*	131.2	5.64/4.15/3.57/4.53	1.39/1.45/1.17	11.64/8.43/9.67	
		ME	*CFE043-TAGW2-CAPS*	132.3	3.86	0.9616	6.09	0.18
	QTkw5A-17	ME	*WPT-8226-XGPW3217*	48.6	5.45	−1.0919	4.15	0.32

Table 2 continued

Traits	QTL	Env[a]	Flanking markers	Position	LOD	A[b]	PVE% (A)[c]	PVE% (AE)[d]
	QTkw5B.1-20	ME	*CFE186-XGWM639*	125.9	4.06	0.8999	3.94	0.46
	QTkw1B.1-47	ME	*WPT-2751-WPT-3465*	113.1	3.59	1.5912	3.03	0.25
L/W	***QKl/w4B.1-7***	E2/E4	*WPT-7569-WPT-3908*	99.1	4.46/7.65	−0.054/−0.0638	12.67/15.41	
		ME	*WPT-7569-WPT-3908*	99.1	4.45	−0.05	7.18	0.10
	QKl/w1D-12	P	*WPT-7946-WPT-6963*	69.7	5.24	0.0280	5.51	
		ME	*WPT-7946-WPT-6963*	69.2	5.02	0.032	2.61	0.24

[a] Indicate the environment in which the QTL was detected. E1, Taian 2008; E2, Taian 2009; E3, Taian 2010; E4, Suzhou 2010; P, the pooled data were derived from the average of the four environments; *ME* multi-environment

[b] Additive effect of the QTL. A positive number indicates that Shannong 01–35 allele is associated with larger trait values than Gaochen9411 allele; a negative number indicates that Gaochen9411 allele is associated with larger trait values than Shannong 01–35

[c] Phenotypic variance explained by additive effects

[d] Phenotypic variance explained by additive-by- environment interaction effects

[e] Bold indicates that QTL with additive effects and AE effects

six loci distributing on chromosomes 3D, 5B, 5A, 5D, 6A, and 7D, respectively. The epistatic interactions between *QKl/w5B.2-14* and *QKl/w6A.1-28* had the most substantial effect by explaining about 10.71 % of the phenotypic variance.

These results suggested that epistatic interactions occurred between the loci on the same or different chromosomes. Epistasis also contributed to the variation of kernel traits, and more attention should be paid to it.

Genotype-by-environment (G × E) interactions for kernel traits

G × E interactions play an important role in determining the adaption and fitness of the genotypes to environment, which were investigated in the present study by evaluating the same set of genotypes in multi-environment (ME).

Additive-by-environment (AE) interactions were observed for all these kernel traits (Table 2). For KL, two additive QTLs (*QKl1B.1-47* and *QKl6A.1-29*) showed AE effects with PVE% of 0.16 and 0.53 %. Three of the four additive QTLs for KW (*QKw5B.1-12*, *QKw6A.1-29*, *QKw4B.1-7*) were detected with AE interaction effects. For KT, three additive QTLs (*QKt1B.1-47*, *QKt4B.1-7*, and *QKt6A.1-29*) were detected with the contributions of AE interactions for phenotypic variations, which were 0.27, 0.47 and 0.46 %, respectively. Two additive QTLs (*QTkw4B.1-7*, *QTkw6A.1-29*) for TKW were found to have AE interaction effects, and accounted for 0.15 and 0.18 % of the phenotypic variation. Both *QKl/w1D-12* and *QKl/w4B.1-7* were detected with AE effects, which explained 0.24 and 0.10 % of the phenotypic variation.

The epistasis-by-environment (AAE) interaction effect was another important component of the GE interaction effects. As shown in Table 3, two pairs of epistatic interactions (*QKw1A.1-19/QKw1A.2-25*, *QKl/w3D-2/QKl/w5A-2*) were found to be involved in AAE effects by accounting for 0.23 and 0.13 % of the phenotypic variation for KW and L/W, respectively. Several additional epistatic interactions for investigated kernel traits were also identified in multi-environment.

Conditional QTL mapping

In additional to unconditional QTLs, a total of 11 conditional QTLs for TKW were detected in conditional QTL analysis, explaining 6.04–17.64 % of the

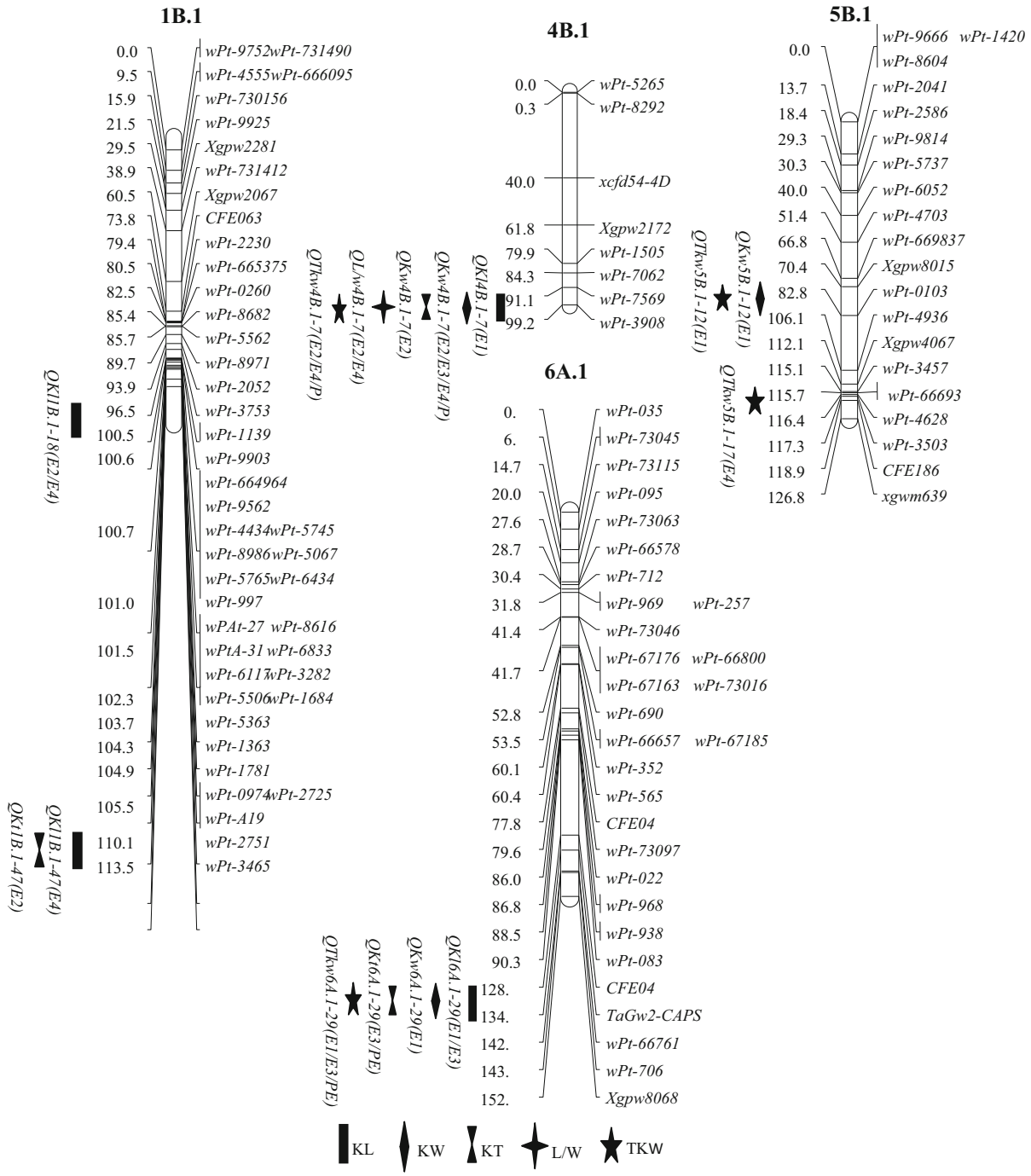

Fig. 1 Genetic linkage map and location of QTL for thousand kernel weight and kernel size traits on chromosomes 1B, 4B, 5B, and 6A based on RIL population. The positions of the markers are listed to the right of the corresponding chromosomes. Map distances are listed on the left chromosomes. QTL are identified on the *left side* of each chromosome. *Different shapes* represent corresponding kernel traits described at the *bottom* of figure

phenotypic variation (Table 4). Of these, *QTkw4B.1-7*, *QTkw6A.1-29*, *QTkw5B.1-12,* and *QTkw5B.1-17* were also identified in unconditional analysis.

Three conditional QTLs were detected when TKW was conditioned on KL. Among them, *QTkw4B.1-7* was detected in all the environments with an increased

Table 3 Epistatic interaction (AA) and epistatic-by-environment interaction (AAE) effects of QTLs for TKW and kernel size

Trait	ENV[a]	QTL[b]	Flanking markers	Position	QTL	Flanking markers	Position	LOD	AA[c]	PVE% (AA)[d]	PVE% (AAE)[e]
KL	E3	*QKl2A-6*	*WPT-665330-XGWM47.1*	132.6	*QKl7A-1*	*WPT-667038-WPT-0961*	6.0	5.06	0.0926	10.33	
		QKl5B.1-13	*WPT-4936-XGPW4067*	107.1	*QKl5B.2-13*	*WPT-7665-WPT-3569*	87.2	4.69	−0.1002	10.61	
	P	*QKl2A-6*	*WPT-665330-XGWM47.1*	132.6	*QKl7A-1*	*WPT-667038-WPT-0961*	5.0	6.58	0.0946	9.22	
		QKl2A-7	*XGWM47.1-XGWM294*	159.1	*QKl7A-4*	*WPT-6768-WPT-6495*	29.3	3.75	−0.0869	2.17	
	ME	*QKl2A-4*	*XGWM425-WPT-3114*	121.8	*QKl5B.1-19*	*WPT-3503-CFE186*	118.3	5.54	0.0716	4.61	0.08
		QKl2A-8	*XGWM294-XGWM614*	189.3	*QKl3B.1-9*	*WPT-5704-WPT-667891*	115.9	4.65	0.3253	8.71	0.08
		QKl5A-2	*WPT-3334-WPT-8226*	10.0	*QKl6A.1-4*	*WPT-731153-WPT-0959*	18.7	6.12	−0.0941	4.21	0.01
		QKl2D-12	*WPT-667485-WPT-1068*	26.8	*QKl7A-10*	*WPT-3393-WPT-7299*	135.2	3.68	−0.0676	4.21	0.95
KW	E2	*QKw1A.1-19*	*WPT-6005-WPT-730172*	141.9	*QKw1A.2-25*	*WPT-667155-WPT-665545*	112.5	5.95	0.1280	6.58	
	ME	***QKw1A.1-19***	***WPT-6005-WPT-730172***	**131.9**	***QKw1A.2-25***	***WPT-667155-WPT-665545***	**123.4**	**3.25**	**0.066**	**2.14**	**0.23**
	E1	*QKw2A-4*	*XGWM425-WPT-3114*	126.8	*QKw7B-16*	*WPT-5343-WPT-4600*	122.1	5.31	0.0898	8.94	
	ME	*QKw1D-12*	*WPT-7946-WPT-6963*	53.7	*QKw2B-1*	*WPT-4916-WPT-0100*	2.3	5.06	−0.0348	5.46	0.18
		QKw2D-12	*WPT-667485-WPT-1068*	26.8	*QKw4B.1-2*	*WPT-8292-XCFD54-4D*	16.3	4.39	0.0465	3.86	0.12
		QKw4A-14	*WPT-6603-WPT-4680*	160.6	*QKw5B.1-11*	*XGPW8015-WPT-0103*	80.4	5.63	0.0366	5.03	0.32
TKW	E2	*QTkw1A.1-19*	*WPT-6005-WPT-730172*	141.9	*QTkw1A.2-25*	*WPT-667155-WPT-665545*	112.5	6.84	2.6836	9.17	
	E3	*QTkw1A.1-11*	*WPT-2976-WPT-2406*	104.8	*QTkw1A.2-28*	*WPT-672089-WPT-730213*	117.4	4.39	1.0450	7.8	
		QTkw1A.1-19	*WPT-6005-WPT-730172*	137.9	*QTkw1A.2-28*	*WPT-672089-WPT-730213*	117.4	6.36	1.5664	6.79	
	P	*QTkw1A.1-19*	*WPT-6005-WPT-730172*	141.9	*QTkw1A.2-25*	*WPT-667155-WPT-665545*	112.5	5.17	2.1313	7.48	
	ME	*QTkw7B-18*	*WPT-4814-WPT-6156*	125.4	*QTkw7D-18*	*WPT-663734-WPT-664384*	47.8	6.03	1.2663	4.05	0.16
		QTkw2D-12	*WPT-667485-WPT-1068*	26.8	*QTkw4B.1-2*	*WPT-8292-XCFD54-4D*	14.3	5.34	1.6842	3.77	0.08
		QTkw7A-18	*XGPW2139-WPT-7151*	223.6	*QTkw7B-20*	*WPT-8938-WPT-1826*	145.6	4.96	−0.5392	3.03	0.10
L/W	E3	*QKl/w5B.2-14*	*WPT-3569-WPT-1348*	90.0	*QKl/w6A.1-28*	*WPT-0832-CFE043*	90.3	5.48	−0.0444	10.71	
		QKl/w5D-7	*WPT-1197-WPT-666937*	166.7	*QKl/w7D-1*	*WPT-3923-WPT-5674*	13.5	4.54	0.0369	8.86	
	P	*QKl/w3D-2*	*WPT-7705-WPT-6909*	28.2	*QKl/w5A-2*	*WPT-3334-WPT-8226*	48.4	3.29	0.0293	5.78	
	ME	***QKl/w3D-2***	***WPT-7705-WPT-6909***	**27.2**	***QKl/w5A-2***	***WPT-3334-WPT-8226***	**47.2**	**3.36**	**0.0424**	**2.18**	**0.13**

[a] See in Table 4

[b] Epistatic interaction effects of QTLs

[c] Phenotypic variance explained by epistatic interaction effects

[d] Phenotypic variance explained by epistatic-by-environment interaction effects

[e] Bold indicates that QTL with epistatic effects and AAE effects

Table 4 Conditional QTLs for thousand kernel weight with respect to kernel size

QTL	Maker interval	Unconditional QTL (Env/PVE% (A))[a]	Conditional QTL [Env/PVE% (A)][b]			
		TKW	TKW|KL	TKW|KW	TKW|KT	TKW|L/W
QTkw4B.1-7	*WPT-7569-WPT-3908*		E1/17.76d			
		E2/8.61	E2/9.16a			
			E3/14.12d			E3/10.25b
		E4/8.66	E4/13.53b			
		P/8.72	P/17.64 b			
QTkw6A.1-29	*CFE043-TAGW2-CAPS*	E1/11.64c				
		E3/8.43c				
		P/9.67	P/10.95 b	P/10.81b		
QTkw5B.1-12	*WPT-0103-WPT4936*	E1/10.57			E1/8.7b	
QTkw5B.1-17	*WPT-666939-WPT-4628*	E4/9.89	E4/9.05a			E4/13.04b
QTkw1B.1-18	*WPT-3753-WPT-1139*			E4/7.18d		E3/6.25d
QTkw1D-15	*WPT-665480-WPT-665204*				E4/6.88d	
QTkw4D-1	*XGPW3113-XGPW342*			E2/8.51d	E1/11.78d	
QTkw5A-3	*WPT-8226-XGPW3217*				E2/11.09d	E4/8.76d
QTkw5B.1-20	*CFE186-XGWM639*		E4/9.05d	E4/10.39d		
QTkw5B.2-9	*WPT-3922-WPT-0484*				E2/6.04d	
QTkw6D-10	*WPT-666615-WPT-666008*			E1/8.33d		E1/7.58d

[a] E and the number before the slash indicate the environment in which the unconditional QTL was detected (E1, Taian 2009; E2, Taian 2010; E3, Taian 2011; E4, Suzhou 2011; P, the data were derived from the average of the four environments); the number after the slash is the percentage of phenotypic variance explained by the corresponding additive effects of the mapped QTL

[b] Before the slash indicates the environment in which the conditional QTL was detected; the number after the slash is the percentage of phenotypic variance explained by the corresponding additive effects of the QTL; the following letter a denotes conditional QTL have no statistical differences in PVE% to that of the unconditional QTL; b denotes a conditional QTL with increase or decrease PVE% to that of the unconditional QTL; c denotes the unconditional QTL couldn't be detected in conditional analysis; d denotes additional QTL in conditional analysis

additive effect in Suzhou 2010 and environment means, explaining 13.53 % (unconditional PVE% = 8.66 %) and 17.64 % (unconditional PVE% = 8.72 %) of the phenotypic variation, respectively. In Taian 2009, *QTkw4B.1-7* was detected with a statistically equal PVE% to that of its corresponding unconditional QTL. *QTkw6A.1-29* was identified with an increased PVE%, accounting for 10.95 % of the phenotypic variation. *QTkw5B.1-17* was found to have no statistical differences in PVE% to that of the corresponding unconditional QTL, indicating that this QTL for TKW was not related to KL.

When the influence of KW on TKW was excluded, only *QTkw6A.1-29* was detected, explaining 10.81 % of the phenotypic variation and showed a higher PVE% compared to that of corresponding unconditional QTL.

QTkw5B.1-12 explained 8.7 % of the phenotypic variation when TKW conditioned on KT and showed a decreased PVE% when compared with corresponding unconditional QTL (unconditional PVE% = 10.57 %), indicating the additive effects of this QTL for TKW were partially contributed by the effects of KT.

Regardless of the L/W effect on TKW, *QTkw5B.1-17* (PVE% = 13.04 %) was detected with an increased PVE% compared with corresponding unconditional QTL (unconditional PVE% = 9.89 %).

Moreover, seven QTLs for TKW were only detected in conditional QTL analysis due to the interference of related kernel trait. When TKW was conditioned on KL, *QTkw5B.1-20* was detected with PVE% of 9.05 %. When the influence of KW on TKW was excluded, *QTkw1B.1-18*, *QTkw4D-1*, *QTkw5B.1-20,* and *QTkw6D-10* were detected with PVE% ranging between 8.33 and 10.39 %. Four QTLs were detected when TKW was conditioned on KT, of which *QTkw4D-1* and *QTkw5A-3* were major QTLs,

accounting for 11.78 and 11.09 % of the phenotypic variation, respectively. Among these extra conditional QTLs, some were affected by one or more related traits. For example, *QTkw1D-15* and *QTkw5B.2-9* were detected when TKW was conditioned on KT; *QTkw4D-1* was detected when conditional analysis was conducted on KW and KT.

Discussion

Additive main effect of QTLs and epistatic interactions between QTLs

One of the most important results of the present study was the characterization of the genetic components that control the expression of the kernel-related traits, including main additive effect QTLs, epistatic interaction QTLs, as well as their environmental interactions. Compared with previous studies that only detected the additive effects of individual QTL (Marza et al. 2006; Araki et al. 1999; Börner et al. 2002; Groos et al. 2003; Huang et al. 2004), novel genetic components for kernel traits were identified in the present study.

In the present study, the locus 4B.4-17 within the genomic region *WPT-7569-WPT-3908* was detected in multiple environments, which was related to KL, KW, KT, TKW, and L/W by explaining 8.61–15.41 % of the phenotypic variation. Another major QTL (*CFE043-TAGW2-CAPS*) on chromosome 6A was found to be associated with KL, KW, KT, and TKW, and explained about 8.43–14.4 % of the phenotypic variation. Six of nine additive QTLs were major QTLs and explained over 10 % of the phenotypic variation, of which *QKt6A.1-29* explained the highest phenotypic variation (16.85 %). These data indicated that the additive effects were the important determinants for each of the kernel trait.

Epistatic interactions are another important factor for understanding the genetic mechanism underlying of complex quantitative traits (Yu et al. 1997; Ma et al. 2005). Even though epistatic effects were not significant for target traits, they could influence the identification of individual QTL, and the efficiency and accuracy of marker-assisted breeding (Ma et al. 2007; Zhang et al. 2008). In our study, twelve pairs of epistatic interactions were identified for the measured kernel traits except KT, while none of the additive QTLs was involved in epistatic interaction effects. For example, the loci 1A.1-19 and 1A.2-25 have not additive effects, but epistatic interaction effects for KW and TKW were detected between them. Epistatic effects were detected between closely linked QTLs or between QTLs on different chromosomes. *QTkw1A.1-19* showed interaction effects with *QTkw1A.2-25* and *QTkw1A.2-28*, and *QTkw1A.2-28* also showed interaction effects with *QTkw1A.1-11*, indicating that some loci may not have significant effects on traits alone but may affect TKW though epistatic interactions with other loci on the same chromosome. Epistatic interaction effects between three QTLs on chromosome 2A as well as three different QTLs on chromosome 7A were detected (*QKw2A-4/QKw7A-16*, *QKl2A-6/QKl7A-1*, *QKl2A-7/QKl7A-4*), suggesting that QTLs for KL and KW were also distributed on chromosomes 2A and 7A.

Additive-by-environment interactions effects (AE) revealed the ability of QTLs to adapt to the environment (Xing et al. 2002). *QTkw4B.1-7* with obvious additive effects was detected in E2, E3, and E4, but its AE interactions only contributed about 0.15 % of the phenotypic variation. Previous studies also reported that QTL had obvious additive effects and was always genetically stable (Zhuang et al. 1997; Moncada et al. 2001). In the present study, only two pairs of epistatic interactions (*QKw1A.1-19/QKw1A.2-25* and *QKl/w3D-2/QKl/w5A-2*) were found with AAE effects ($P < 0.001$). Zhuang et al. (1997) detected some epistatic QTLs with AAE interactions, whereas Xing et al. (2002) failed to detect any epistatic QTLs with AAE interactions in multiple environments. This may be due to that the effects of epistatic interactions are usually small, and their environmental effects may have been too small to be detected.

Above analyses indicated that in addition to the major additive effects, more attentions should be paid to the effects of epistatic interactions and G × E interactions in wheat kernel size improvement. The results obtained in this study have meaningful implications in wheat breeding programs.

Comparison conditional and unconditional QTLs for TKW

Complex genetic relationships among TKW and various other kernel traits were largely unexplored under unconditional analysis. However, conditional

analysis has the ability to discern contribution of each component trait to a complex trait. In the present study, conditional QTL mapping was employed to investigate the genetic contribution of kernel size on QTL expression of TKW. Four outcomes can be obtained upon comparison of the effects of unconditional and conditional QTL mapping (Zhao et al. 2006; Ye et al. 2009): (a) a QTL detected with a similar or equal effect, indicating that this QTL for TKW is independent of a given trait; (b) a QTL detected with either a greatly reduced or enhanced effect, indicating that this QTL for TKW is partially influenced by related traits; (c) a QTL cannot be identified, meaning that this QTL is completely controlled by conditional traits; and (d) an additional QTL can be detected, suggesting that this QTL is entirely suppressed by conditional traits.

Based on the results of conditional and unconditional QTL mapping, *QTkw4B.1-7* was partially contributed by KL and L/W, and entirely contributed by KW and KT; *QTkw6A.1-29* was found entirely due to variation of KL, KW, KT, and L/W; QTkw5B.1-12 was partially contributed by KT and entirely contributed by KL, KW, and L/W; *QTkw5B.1-17* was found to be primarily dependent on variation of KW and KT, and in part with the variation of L/W, and independent of KL. The results described above demonstrated that QTLs for TKW were contributed by KL, KW, KT, and L/W, and KW had the strongest contribution on TKW at the QTL level, which further confirmed the correlation analysis between TKW and kernel size (Table S2). These results are consistent with previous studies (Dholakia et al. 2003; Sun et al. 2009). Seven additional QTLs for TKW were detected by conditional analysis, suggesting that these QTLs were completely suppressed by kernel size traits. One possible explanation for these additional QTLs was the genes with very small genetic effects and undetected by unconditional analysis. Notably, the contributions of kernel size traits to TKW were different at the QTL level because of the great influence of the environment. For example, *QTkw6A.1-29* was found to be entirely due to variation of KW and KT, but KL had different contributions to TKW in different environments.

In the present study, comparison results of the unconditional and conditional QTL analyses showed that these kernel size traits all had effects on the expression of QTLs for TKW, and the increase in TKW was driven primarily by KW. The combination of conditional and unconditional QTL mapping in the present study provided an opportunity for the detection of the mechanism underlying TKW of wheat.

Important QTL clusters associated with kernel traits

Although a large number of QTL analyses for TKW and kernel size have been reported, most of them only focused on the additive effects using unconditional QTL mapping method without considering epistatic and G × E interaction effects of QTLs. In this report, additive and epistatic interaction QTLs as well as their environmental interactions effects for kernel traits were analyzed. In addition, we combined conditional and unconditional QTL analyses to detect the molecular mechanism governing TKW. Based on the above analyses, three important QTL clusters were identified with high PVE% and good stability, which distributed on chromosomes 4B, 5B, and 6A (Fig. 1).

Several QTLs for TKW and kernel size have been reported on chromosome 6A (Huang et al. 2006; Sun et al. 2009; Gegas et al. 2010). In present study, *QTkw6A.1-29* (*CFE043-TAGW2-CAPS*) was identified using both unconditional and conditional QTL mappings, which also co-located with *QKl6A.1-29*, *QKw6A.1-29,* and *QKt6A.1-29*. Notably, this QTL was located near the region of *TaGW2*, one important gene associated with TKW and KW located on chromosome 6A (Su et al. 2011). The results confirmed that the region near *TaGW2* of chromosome 6A may harbor a robust QTL cluster for kernel-related traits, which should be paid more attention in breeding programs.

QTkw4B.1-7 was located on the short arm of chromosomes 4B and shared the same interval with *QKw4B.1-7*, *QKl4B.1-7 QKt4B.1-7*, and *QKl/w4B.1-7*. The results of conditional QTL mapping also indicated that *QTkw4B.1-7* was partially contributed by KL, and entirely explained by KW. Few major QTLs for TKW have been located on chromosome 4B in previous studies, among them *Qgw4B-15* (*gwm192a-gwm192b*) and *QTkw.macs-4B.2* (*XRht-B1–Xgwm368*) were found to be associated with dwarfing gene *Rht-B1* (Zhang et al. 2013; Patil et al. 2013); *QTgw.crc-4B, QTgw.wa-4BL.e2, and QTkw-pk.cimmyt-4BL* were located on the long arm of chromosome 4B (Huang et al. 2006; Wang et al. 2011;

Hao et al. 2014). In addition, several QTLs for kernel size on chromosome 4B were reported by Sun et al. (2009) and Gegas et al. (2010). By comparing the position of marker intervals, *QTkw4B.1-7* was considered preliminarily as a new QTL for its large distance from reported QTLs. Based on the above, we have developed a new CAPS marker, which associated is with TKW and kernel size, and can be used in genetic map construction and marker-assisted selection (Chen et al. 2014).

QTkw5B.1-12 shared the same interval (*WPT-0103-WPT-4936*) with *QKw5B.1-12*, and both were found with high PVE%. Through conditional analysis, we found that KL, KW, and L/W completely contributed to *QTkw5B.1-12*. A number of previous reports have identified QTLs for TKW and kernel size on chromosome 5B (Groos et al. 2003; Breseghello and Sorrells 2006; Ramya et al. 2010). A comparison of the QTLs detected in the present study to that identified in other studies showed that these important QTLs were located at an approximately equivalent or adjacent chromosomal region.

In conclusion, we found three important QTL clusters that associated with TKW and kernel size by conditional and unconditional analyses. These QTLs would be of great value for marker-assisted selection in wheat breeding. The combination of unconditional and conditional QTL mapping methods in the current study can evaluate genetic relationships between TKW and kernel size at an individual QTL level. In addition, the present study provides new insights into the understanding of the genetic mechanisms and regulation networks of a complex trait.

Acknowledgments This work was supported by the "National Natural Science Foundation of China" (31171554), the "Natural Science Foundation of Shandong" (ZR2015CM036), the "Creation of Wheat Germplasm in Shandong Province project", and the "Research fund for the doctoral program of higher education in China" (No: 20123702110016).

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