RESEARCH ARTICLE

Wheat kernel dimensions: how do they contribute to kernel weight at an individual QTL level?

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Abstract

Kernel dimensions (KD) contribute greatly to thousand-kernel weight (TKW) in wheat. In the present study, quantitative trait loci (QTL) for TKW, kernel length (KL), kernel width (KW) and kernel diameter ratio (KDR) were detected by both conditional and unconditional QTL mapping methods. Two related $F_{8:9}$ recombinant inbred line (RIL) populations, comprising 485 and 229 lines, respectively, were used in this study, and the trait phenotypes were evaluated in four environments. Unconditional QTL mapping analysis detected 77 additive QTL for four traits in two populations. Of these, 24 QTL were verified in at least three trials, and five of them were major QTL, thus being of great value for marker assisted selection in breeding programmes. Conditional QTL mapping analysis, compared with unconditional QTL mapping analysis, resulted in reduction in the number of QTL for TKW due to the elimination of TKW variations caused by its conditional traits; based on which we first dissected genetic control system involved in the synthetic process between TKW and KD at an individual QTL level. Results indicated that, at the QTL level, KW had the strongest influence on TKW, followed by KL, and KDR had the lowest level contribution to TKW. In addition, the present study proved that it is not all-inclusive to determine genetic relationships of a pairwise QTL for two related/causal traits based on whether they were co-located. Thus, conditional QTL mapping method should be used to evaluate possible genetic relationships of two related/causal traits.

[Cui F., Ding A., Li J., Zhao C., Li X., Feng D., Wang X., Wang L., Gao J. and Wang H. 2011 Wheat kernel dimensions: how do they contribute to kernel weight at an individual QTL level? *J. Genet.* **90**, 409–425]

Introduction

Wheat (*Triticum aestivum* L.) is a major food crop worldwide, and high yield is a predominant objective in breeding programmes. Kernel weight, one of the three major yield components, is greatly influenced by kernel dimensions (KD), such as kernel length (KL), kernel width (KW), etc. Therefore, it is of utmost interest to obtain more information about the underlying genetic control of KD traits.

With the rapid development of molecular marker technology in wheat, increasing numbers of QTL studies have been conducted in an attempt to dissect the genetic basis of thousand-kernel weight (TKW), and all the 21 wheat chromosomes have now been proven to harbour factors affecting it (Halloran 1976; Giura and Saulescu 1996; Araki *et al.* 1999; Shah *et al.* 1999; Kato *et al.* 2000; Varshney *et al.* 2000; Ammiraju *et al.* 2001; Zanetti *et al.* 2001; Böner *et al.* 2002; Campbell *et al.* 2003; Groos *et al.* 2003; Huang *et al.* 2003, 2004, 2006; McCartney *et al.* 2005; Verma *et al.* 2005; Kirigwi *et al.* 2007; Li *et al.* 2007; Hai *et al.* 2008; Röder *et al.* 2008; Golabadi *et al.* 2010; McIntyre *et al.* 2010; Su *et al.* 2010; Zheng *et al.* 2010). To obtain information about genetic relationships between TKW and KD at the QTL level, some researchers performed QTL detection for both TKW and KD simultaneously in the same biparental mapping populations (Campbell *et al.* 1999; Dholakia *et al.* 2003; Breseghello and Sorrells 2007; Sun *et al.* 2009; Ramya

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Keywords. wheat; kernel dimensions; thousand-kernel weight; conditional QTL mapping; genetic relationship.

et al. 2010; Tsilo *et al.* 2010). In these studies, a common problem associated with the analyses of the data reported so far is that the analysis of QTL for TKW and its causal traits was conducted based on the phenotypic values separately. Generally, they determined genetic associations of two QTL for pairwise traits based on whether they were co-located. Due to the interference of other traits, it should be difficult to clarify the actual relationships among these traits precisely and comprehensively at the QTL level (Ye *et al.* 2009).

More recently, a method for multivariate conditional analysis was proposed for analysing the contributions of component traits to a complex trait and for investigating the genetic relationship between two traits at the QTL level (Wen and Zhu 2005). Based on this methodology, it is possible to reveal that the gene expression for a complex trait may be contributed by its different causal genes expression at different levels, thus helping us in understanding the nature of component traits in determining the phenotypic value of a complex trait. To our knowledge, few reports of QTL analysis based on this methodology have been reported (Guo *et al.* 2005; Zhao *et al.* 2006; Mei *et al.* 2007; Li *et al.* 2008; Liu *et al.* 2008a; Ye *et al.* 2009; Cui *et al.* 2011). However, none of them considered TKW and its components in wheat. In view of the strong positive correlations between TKW and KD, it will be of great value to probe their genetic control system and to evaluate their genetic relationships on individual loci by conditional QTL mapping method.

Population size has a great effect on the estimation of QTL number and genetic effects (Beavis 1998; Mackay 2001; Bernardo 2004; Schön *et al.* 2004; Vales *et al.* 2005; Zou *et al.* 2005; Buckler *et al.* 2009). The precision and efficiency of QTL detection will be enhanced by combining at least two related populations (Breseghello and Sorrells 2007; Kumar *et al.* 2007; Ma *et al.* 2007; Buckler *et al.* 2009; Uga *et al.* 2010; Gegas *et al.* 2010). In the present study, we performed QTL detection for TKW, KL, KW and kernel diameter ratio (KDR) based on the combination of two related populations, of which one was a large population with up to 485 lines and the other was smaller comprising 229 lines. Both unconditional mapping methods and conditional mapping methods for multivariate conditional analysis were used. The objectives of this study were to: (i) accurately identify the genetic factors affecting TKW, KL, KW and KDR; (ii) specify the genetic relationships between TKW and KD at individual QTL level; and (iii) discuss the effect of combining two related populations of different size on the efficiency and precision of QTL detection.

Materials and methods

Experimental populations and their evaluation

Two related wheat $F_{8:9}$ recombinant inbred line (RIL) mapping populations were used in this study, which will be referred here as populations 'WJ' and 'WY'. WJ was derived from the cross between Weimai 8 and Jimai 20, comprising 485 lines. WY consisted of 229 lines, derived from the cross between Weimai 8 and Yannong 19. The common parent Weimai 8 is a large-spike type of the ideotype model and was released by Weifang Municipal Academy of Agricultural Sciences, Shandong, China, in 2003; Jimai 20 and Yannong 19, two superior quality wheat varieties, are multipanicle types, and they were released by Crop Research Institute, Shandong Academy of Agricultural Sciences, China, in 2003, and by Yantai Municipal Academy of Agricultural Sciences, Shandong, China, in 2001, respectively. Among these three parental varieties, Weimai 8 has the highest TKW and the largest kernels (table 1). In addition, the common parent Weimai 8 is a 1BL/1RS translocation line whereas the other two parents have a common 1B chromosome. The parents together with the RILs, were planted in four environments in Shandong Province, China; Tai'an in 2008–2009 (E1), and Tai'an in 2009–2010 (E2), Zao'zhuang in 2009–2010 (E3) and Ji'ning in 2009–2010 (E4). This study sites at Tai'an (36°09′N, 117°09′E, altitude 128 m), Zao'zhuang (36°50′N, 117°33′E, altitude 65 m) and Ji'ning (35°27′N, 116°35′E, altitude 37 m) represent three different wheat-growing agroclimatic regions. The temperature in winter was lower in Tai'an in 2008 than 2009; and the wind in June was stronger in Tai'an in 2009 than 2010. A two-row plot with rows 2-m long and 30 cm apart was used, and 50 seeds were planted in each row. Since large variation in plant height existed among RIL populations in both WJ and WY (Cui *et al.* 2011), the RIL populations were planted in adjacent plots according to plant heights to avoid severe shading by adjacent plants which could affect a shorter plant surrounded by tall plants. The RILs together with parents were sown on 7th October 2008 for E1, 8th October 2009 for E2, 11th October 2009 for E3 and 17th October for E4, respectively. Concerning anthesis data, small variation existed among RIL populations in both WJ and WY, the range being about five days. All recommended agronomic practices were followed in all the four experiments, and all the experimental fields had loamy soil. However, gibberellic disease was very serious in Zao'zhuang in 2009–2010 in the watery stage and milk ripe stage. Kernel traits were evaluated after harvest. Seed was thoroughly cleaned and all nonwheat materials and broken kernels were removed before trait evaluation. TKW was evaluated in grams by weighing two samples of 1000 kernels from each plot. Two samples of 20 kernels from each plot were lined up length-wise along a ruler with a precision of 0.1 mm, to measure KL, and then the kernels were arranged breadth-wise to measure KW. All lengths were reported in centimetres. The KDR was calculated as KDR = KL/KW.

Analysis of molecular and biochemical markers

Molecular markers of G-SSR, EST-SSR, ISSR, STS, SRAP and RAPD were used to genotype the three parents and their derived lines. Of these, relevant information

Table 1. Phenotypic values for thousand-kernel weight and kernel dimensions of three parents and two RIL populations in four growing environments in wheat.

Trait[a]	En.[b]	Parent			WJ[c]					WY[c]				
		Weimai 8	Jimai 20	Yannong 19	Mean	SD	Min–max	Skewness	Kurtosis	Mean	SD	Min–max	Skewness	Kurtosis
TKW (g)	E1	49.11	28.53	44.68	40.95	5.504	19.59–55.33	−0.203	0.6442	41.68	5.039	23.41–51.90	−0.731	0.582
	E2	50.46	32.43	42.88	39.45	4.843	23.89–53.96	0.133	0.041	40.56	3.842	30.95–53.12	0.031	0.172
	E3	35.41	28.70	28.71	30.37	4.26	16.36–42.34	−0.166	−0.244	30.67	4.049	19.22–42.65	0.088	0.022
	E4	49.00	30.12	41.28	43.09	19.16	25.00–54.57	−0.244	0.559	42.60	4.665	30.58–56.89	−0.112	−0.241
KL (cm)	E1	0.707	0.667	0.680	0.666	0.032	0.588–0.753	0.109	−0.248	0.690	0.033	0.620–0.780	0.369	−0.130
	E2	0.710	0.637	0.680	0.642	0.033	0.530–0.752	0.277	0.311	0.664	0.037	0.562–0.805	0.281	0.740
	E3	0.645	0.575	0.631	0.603	0.037	0.450–0.700	−0.637	1.658	0.629	0.031	0.555–0.751	0.692	1.145
	E4	0.680	0.641	0.676	0.645	0.034	0.465–0.765	−0.329	1.723	0.667	0.036	0.570–0.765	0.053	−0.241
KW (cm)	E1	0.360	0.313	0.327	0.332	0.020	0.247–0.380	−0.554	1.182	0.330	0.019	0.240–0.373	−0.625	1.447
	E2	0.360	0.299	0.320	0.324	0.023	0.251–0.400	−0.040	0.402	0.322	0.020	0.265–0.396	0.032	0.504
	E3	0.333	0.281	0.295	0.314	0.027	0.250–0.490	2.20	11.67	0.307	0.018	0.258–0.366	−0.019	0.213
	E4	0.363	0.330	0.318	0.342	0.018	0.275–0.395	−0.258	0.487	0.336	0.019	0.266–0.395	−0.226	0.654
KDR	E1	1.963	2.128	2.082	2.011	0.143	1.691–2.756	0.998	2.980	2.098	0.141	1.788–2.610	0.469	0.561
	E2	1.972	2.130	2.125	1.991	0.142	1.560–2.498	0.308	0.410	2.071	0.161	1.744–2.548	0.385	−0.197
	E3	1.940	2.048	2.139	1.941	0.169	1.010–2.470	−1.259	7.809	2.058	0.149	1.764–2.612	0.777	0.493
	E4	1.874	1.944	2.126	1.889	0.120	1.606–2.301	0.476	0.234	1.991	0.156	1.603–2.496	0.399	−0.037

SD, standard deviation. [a]TKW, thousand-kernel weight; KL, kernel length; KW, kernel width; KDR, kernel diameter ratio.
[b]E1, E2, E3 and E4 represent the environments of 2008–2009 in Taian, 2009–2010 in Taian, 2009–2010 in Zaozhuang and 2009–2010 in Jining, respectively.
[c]WJ and WY represent the populations derived from the cross between Weimai 8 and Jimai 20 and between Weimai 8 and Yannong 19, respectively.

regarding G-SSR markers, including BARC, CFA, CFD, CFT, GWM, GDM, GPW, WMC and PSP codes, as well as PCR-based STS markers of the MAG code, were taken from the GrainGenes web site (http://wheat.pw.usda.gov). Relevant information about EST-SSR markers prefixed CFE, KSUM and CNL are publicly available (http://wheat.pw.usda.gov/ITMI/EST-SSR/). EST-SSR markers of SWES and WW codes were developed and kindly provided by Professor Sishen Li, College of Agronomy, Shandong Agricultural University, China. EST-SSR markers with the prefixes CWEM, EDM and CWM were published in reference articles by Peng and Lapitan (2005), Mullan *et al.* (2005) and Gao *et al.* (2004), respectively. ISSR markers were developed by the University of British Columbia Biotechnology Laboratory (UBCBL) (Nagaoka and Ogihara 1997). Relevant information about chromosome 1RS-specific markers of rye were detailed by Zhao *et al.* (2009), and functional markers, were detailed by Liu *et al.* (2008b) and Liang *et al.* (2010). The differences of high molecular weight glutenin subunits (HMW-GS) at *Glu-a1*, *Glu-b1* and *Glu-d1* between the parents were detected and used as biochemical markers.

Each PCR reaction for G-SSR, EST-SSR and PCR-based STS markers was conducted in a total volume of 25 μL in a TakaRa PCR thermal cycler (Takara, Shiga, Japan) or in a Bio-Rad 9600 thermal cycler (Bio-Rad, California, USA). PCR reaction mixture was compounded according to the formula described by Röder *et al.* (1998). Amplifications were performed using a touchdown PCR protocol detailed by Hao *et al.* (2008). PCR reaction mixture, as well as PCR protocol for SRAP and ISSR markers followed the formula and the procedure detailed by Li *et al.* (2007), and for RAPD markers, by Suenaga *et al.* (2005). The PCR products were separated in 6% nondenaturing polyacrylamide gels. Gels were then silver stained and photographed. Types of HMW-GS were detected by using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) (Singh and Sheperd 1991). Markers of BARC, CFA, CFD, GWM, GDM and WMC codes were also screened on the nullisomic and tetrasomic stocks of Chinese Spring (CSNT) to assign them to chromosomes, where possible.

Construction of the genetic linkage map

Linkage groups were constructed by MAPMAKER 3.0 (Lander *et al.* 1987). First, the 'ANCHOR' command was used to locate marker loci on chromosomes based on the CSNT identification and the public genetic maps in GrainGenes 2.0 (http://wheat.pw.usda.gov/GG2/index.shtml). Then, the assignment of the remaining loci to chromosomes was made using the 'ASSIGN' command at a LOD score of 3.0. Based on the linkage group defined above, JoinMap, version 3.0 (Biometris, Wageningen, The Netherlands; http://www.joinmap.nl), was used to construct the linkage map, and centiMorgan units were calculated using the Kosambi mapping function (Kosambi 1944).

Data analysis and QTL mapping

To estimate the variance components, all the four traits were first analysed with the MINQUE method proposed by Zhu (1992). Phenotypic correlation coefficients between TKW and TKW components (TKWC) were calculated separately for each environment. Basic statistical analysis was implemented by the software SPSS13.0 (SPSS, Chicago, USA). Conditional genetic analysis was conducted based on the phenotypic values of TKW conditioned on each of its component traits, that were obtained by the mixed-model approach (Zhu 1995; Wen and Zhu 2005). Conditional phenotypic values $y_{(TKW|TKWC)}$ indicate the value of TKW without the influences of its component traits.

Both the observed phenotypic values ($y_{(TKW)}$) and the conditional phenotypic values ($y_{(TKW|TKWC)}$) obtained from each environment of E1, E2, E3 and E4, and the pooled data collected from the average of the four environments above (P) were used for QTL mapping analysis. In addition, phenotypic values of KL, KW and KDR obtained from each environment and the pooled data were also used for QTL scan, with a view to analyse pleiotropic effects between TKW and its related traits at the QTL level. QTL screen was conducted using inclusive composite interval mapping by IciMapping 3.0 based on step-wise regression of simultaneous consideration of all marker information (http://www.isbreeding.net/). The walking speed chosen for all QTL was 1.0 cM. The threshold LOD scores were calculated using 1000 per mutations. However, here we ignored the QTL with a LOD value of <2.5 to make the QTL reported authentic and reliable.

Rules for naming QTL

The assignment of a QTL name is named according to the following rules: italic upper case '*Q*' denotes 'QTL'; letters following it are the abbreviation of the corresponding trait; the next upper case letters sandwiched the two dashes '-' indicates the population in which the corresponding QTL was detected; next, a numeral plus an upper case letter, 'A', 'B' or 'D', indicates the wheat chromosome on which the corresponding QTL was detected; if a break occurred on a chromosome, a dash '-' plus a numeral are placed as suffixes to distinguish different segments of the corresponding chromosome; the last numeral after a period denotes the number of environments in which the corresponding QTL was detected; and if the name of two different QTL for the same trait look the same, a lower case letter is used to distinguish them.

Results

Phenotypic variation of traits and correlations with TKW

The parental performance and variation among the two RIL lines for TKW, KL, KW and KDR in four environments are shown in table 1. Over all in four environments, the significant differences of all the four traits existed at the 0.05 level between Weimai 8 and Jimai 20, and between Weimai 8 and Yannon 19. Weimai 8 was characterized by higher TKW and larger kernels, compared to both Jimai 20 and Yannong 19. In addition, both RIL lines and parents had the lowest phenotypic values for both TKW and KD in E3 among all the four trials in both WJ and WY. This might be due to the outbreak of gibberellic disease in Zao'zhuang in 2009–2010 in the watery stage and milk ripe stage. The phenotypic variations of all the four traits among the RIL lines were obvious in both populations and segregated continuously. Both absolute values of skewness and kurtosis for TKW were less than 1.0 in all the four trials in both WJ and WY, indicating a normal distribution. The absolute values of skewness for KL, KW and KDR were less than 1.0, with the exception of KW in E3 and KDR in E3. The absolute values of skewness for KL were less than 1.0 in E1 and E2, as that of KW in E2 and E4 and of KDR in E2 and E4. The results indicate that all the four traits were typically quantitative traits controlled by a few minor or major genes and that the data are suitable for QTL analysis. In addition, strong transgressive segregations were observed in all the four traits in all environments, indicating that alleles with positive effects are distributed among the parents. The evaluation of the phenotypic correlations between TKW and KL, KW and KDR are listed in table 2. Positive and significant correlations were observed between TKW and KL, and between TKW and KW, in both WJ and WY in all environments, whereas negative significant correlations were observed between TKW and KDR. The highest correlation coefficients in absolute were observed between TKW and KW, and the lowest were between TKW and KDR. Overall, the results were consistent in four environments in both WJ and WY. These findings indicate a strong stable genetic association between TKW and KW. In both WJ and WY, conditioning TKW on KW led to the strongest reduction of phenotypic variance, while TKW conditioned on KDR showed variances nearly as high as the unconditioned TKW (table 2). These findings indicate that KW contributes to the highest level of TKW phenotypic variation, next to KL, consistent with the results of phenotypic correlation analysis.

Construction of genetic linkage maps

The genetic map constructed based on the WJ population included 344 loci on the wheat chromosomes and spanned 2855.5 cM, with an average density of one marker per 8.30 cM. There were six linkage gaps with linkage distances >50 cM (figure 1). Marker distribution ranged from 45 on chromosome 4A to 3 on chromosomes 4D and 7D. The WY population was used to establish a genetic map consisting of 358 loci distributed in 27 linkage groups with six linkage gaps, and it covered 3010.70 cM of the whole genome with an average distance of 8.41 cM between the adjacent loci (figure 1). The number of markers per chromosome ranged from 40 on chromosome 1B to 3 on chromosome 3D. The two linkage maps contained 69 common

Table 2. Phenotypic correlations between thousand-kernel weight and kernel dimensions and the phenotypic variances of thousand-kernel weight and thousand-kernel weight conditioned on kernel dimensions.

	Correlation				Directed and conditioned variances			
Trait	E1	E2	E3	E4	E1	E2	E3	E4
TKW	–/–	–/–	–/–	–/–	30.29/25.39	23.46/14.76	18.15/16.39	19.16/21.76
KL	0.425**/0.380**	0.495**/0.289**	0.368**/0.340**	0.458**/0.331**	24.81/21.73	17.80/13.47	15.07/14.20	15.09/19.65
KW	0.724**/0.617**	0.618**/0.479**	0.555**/0.571**	0.577**/0.510**	14.41/15.73	14.09/11.32	11.84/10.82	12.74/16.32
KDR	−0.378**/−0.288**	−0.273**/−0.189**	−0.275**/−0.237**	−0.111*/−0.141*	25.96/23.28	21.80/14.16	16.45/15.15	18.86/21.62

For each entry, the first figure refers to WJ, and the second to WY. For abbreviations, see table 1.
*Correlation is significant at when $P < 0.01$ level; **correlation is significant at when $P < 0.01$ level; –/–, data not available.

loci. The chromosomal locations and the orders of the markers in the two maps were generally in agreement with published reports in GrainGenes 2.0 (http://wheat.pw.usda.gov/GG2/index.shtml). Positions of the loci common to the two maps were approximately in accordance. In addition, a 1BL/1RS translocation event was confirmed by the linkage maps of chromosome 1B in both WJ and WY. Functional markers and biochemical markers were accurately mapped to their corresponding chromosomes. The recommended map distance for genomewide QTL scanning is an interval length less than 10 cM (Doerge 2002). Thus, the maps were suitable for genomewide QTL scanning in this study.

Unconditional QTL mapping in WJ and WY populations

Thousand-kernel weight: In total, 14 and nine putative additive QTL for TKW were detected in WJ and WY, respectively (see tables 1 and 2 in electronic supplementary material at http://www.ias.ac.in/jgenet/; figure 1). They together covered all the 21 wheat chromosomes except 1B, 1D, 3A, 4B, 4D and 5A. Of these, *QTkw-WJ-4A.5* and *QTkw-WJ-6A.5* were verified in all the five trials, and they individually accounted for 2.36–5.29% and 2.32–5.94% of the phenotypic variance, respectively. *QTkw-WJ-5B.4* was identified reproducibly in four of the five trials, exhibiting 3.35–4.75% of the phenotypic variance. Of the five QTL that showed significance in three trials, *QTkw-WY-2A.3* and *QTkw-WY-2B-1.3* individually accounted for 8.01–12.60% and 8.36–14.79% of the phenotypic variance, respectively, both being major QTL. The remaining 15 QTL showed significance in only two or one of the five trials. Alleles of QTL with increased effect were identified from both parents in both WJ and WY.

Kernel length: QTL mapping detected 12 and seven chromosomal regions governing KL totally in the five trials in WJ and WY, respectively (see tables 1 and 2 in electronic supplementary material; figure 1). These QTL were located on 1A, 1B, 1D, 2A, 2D (2 QTL), 3B, 5A, 5B, 6A and 6D in WJ, and on 3A, 6A (2 QTL), 6B (2 QTL) and 6D (2 QTL) in WY. Of these, only *QKl-WJ-3B.5* was stable across all the five trials, explaining 3.94–8.18% of the phenotypic variance. Two QTL, *QKl-WJ-5B.4* and *QKl-WY-6B.4*, were stable across four of the five trials, individually exhibiting 3.51–6.36% and 7.78–11.64% of the phenotypic variance, respectively. In addition, there were three QTL for KL that showed significance in three of the five trials, all individually accounting for less than 10% of the phenotypic variance. The remaining 13 QTL were significant in only two trials or one, of which, only *QKl-WY-6B.2* was major QTL, accounting for 6.80–12.95% of the phenotypic variance. The additive effects for seven QTL were positive with Weimai 8 increasing the QTL effects in WJ, but Jimai 20 contributed the favourable alleles for all the seven QTL in WY.

Kernel width: For KW, 13 putative additive QTL in WJ and nine in WY were detected, respectively (see tables 1 and 2 in electronic supplementary material; figure 1). They were together assigned to 14 wheat chromosomes, all the 21 wheat chromosomes with the exception of 1B, 4A, 4D, 5B, 6D, 7A and 7D. Of these, only two QTL, *QKw-WJ-5A-3.3* and *QKw-WJ-6A.4*, were reproducibly identified in at least three trials, each exhibiting 5.17–7.83% and 3.45–6.56% of the phenotypic variance, respectively. Of the remaining 20 QTL that were significant in only two trials or one, there were four QTL individually accounting for more than 10% of the phenotypic variance. Favourable alleles for KW were dispersed among the parents in both WJ and WY.

Kernel diameter ratio: Concerning KDR, up to 25 putative additive QTL were detected in two populations, distributed across 13 of the 21 wheat chromosomes (see tables 1 and 2 in electronic supplementary material; figure 1). Of these, *QKdr-WY-6B.5*, explaining 5.24–13.97% of the phenotypic variance, was reproducibly identified in all the five trials. Two QTL, *QKdr-WJ-2A.4* and *QKdr-WJ-5A-1.4*, were stable across four of the five trials, individually exhibiting 2.96–4.19% and 13.49–15.08% of the phenotypic variance, respectively. In addition, there were four QTL that showed significance in three trials, all individually explaining less than 10% of the phenotypic variance. In total, 10 of the 25

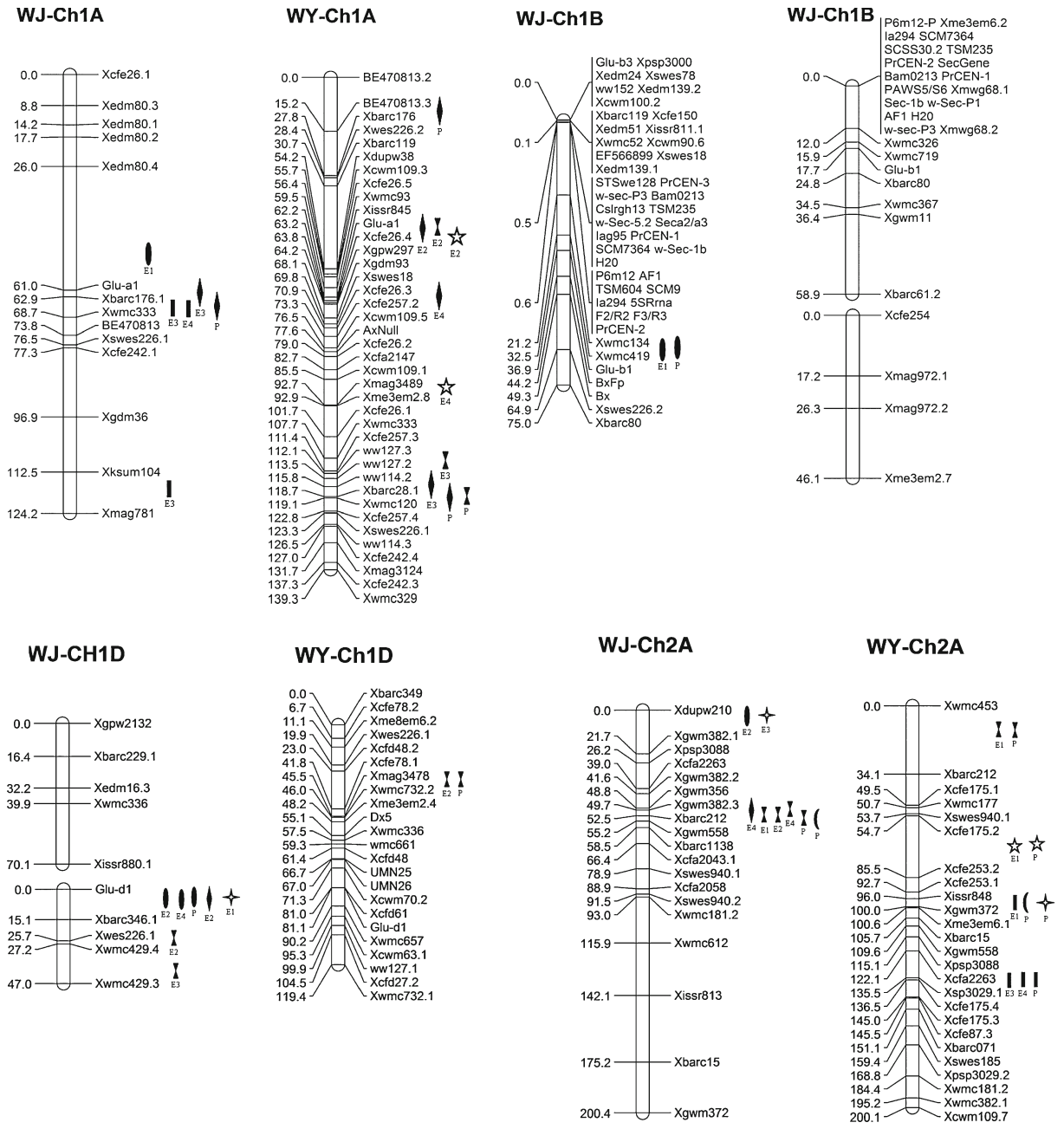

Figure 1. Genetic linkage map and location of putative QTL for thousand-kernel weight and kernel dimensions based on 485 RILs derived from Weimai 8 × Jimai 20 and 229 RILs from Weimai 8 × Yannong 19, with the prefixes WJ and WY, respectively. The positions of marker loci on chromosomes are listed on the left of the corresponding chromosomes. Map distances are shown in centiMorgans and were calculated using the Kosambi (1944) mapping function. A putative QTL with LOD > 2.5 is placed on its corresponding flanking markers. QTL symbols are described at the bottom right of figure 1, and an uppercase letter E plus a numeral, 1, 2, 3 or 4, or the uppercase letter P under the corresponding QTL symbol indicates the trial in which QTL was detected. Here, we only showed the conditional QTL detected in the trial where it was undetectable by unconditional QTL mapping method (figure 1 continues on following pages).

QTL alleles increasing KDR were donated by the common parent Weimai 8 in the two populations.

Conditional QTL mapping in the WJ and WY populations

Of the 14 unconditional additive QTL for TKW in WJ, eight, five and four of them were undetectable when TKW was conditioned on KW, KL and KDR, respectively (table 3). However, one and four conditional QTL showed additive effects similar to that of the corresponding unconditional QTL when TKW was conditioned on KW and KDR, respectively. In the WY population, of the nine putative unconditional additive QTL for TKW, six, two and four failed to show significance, respectively, when the influence of KW,

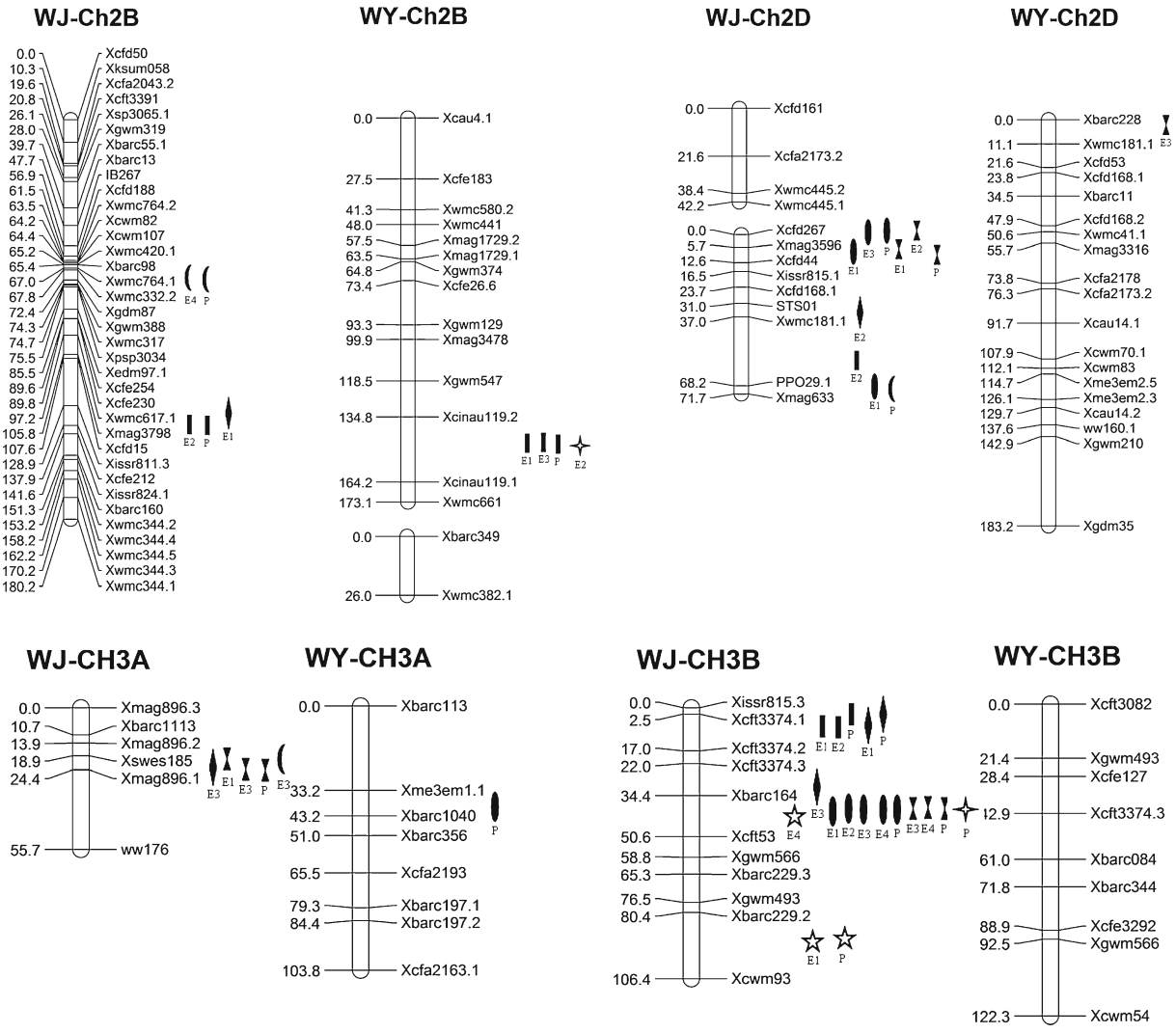

Figure 1 (*contd*)

KL and KDR on TKW was excluded; but zero, two and three, respectively, showed additive effects similar to that of the corresponding unconditional QTL (table 4). In both WJ and WY, changes in additive effects of the remaining conditional QTL, compared to its corresponding unconditional QTL, was inconsistent over trials, being unchanged in one trial, but greatly/moderately changed in another one, or being greatly changed or even undetectable in one trial, but moderately changed in another one.

In addition, conditional QTL mapping analysis revealed numerous additional additive QTL that could not be identified by unconditional QTL mapping method (tables 5 and 6). Ten, seven and 10 additional conditional QTL in WJ, and five, eight and six in WY were detected when TKW was conditioned on KL, KW and KDR, respectively. Of these, two, three and four in WJ, and two, one and three in WY, respectively, have been detected in unconditional QTL mapping analysis in other trial/trials.

Discussion

Kernel dimensions: how do they contribute to the kernel weight at an individual QTL level?

A comparison of conditional and unconditional QTL mapping analysis provides information about the genetic control system involved in the synthetic process between TKW and its related traits at the level of an individual QTL. For example, when performing conditional QTL analysis of TKW conditioned on KL (TKW|KL), there are four possible results: (i) a QTL detected by the unconditional method can be identified with a similar or equal effect, indicating that this QTL for TKW expresses independently for the given trait KL; (ii) a QTL detected by the unconditional method can be identified with either a greatly reduced or a greatly enhanced effect, suggesting that this QTL for TKW is partially, but not completely, associated with KL; (iii) a QTL detected by the unconditional method cannot be identified,

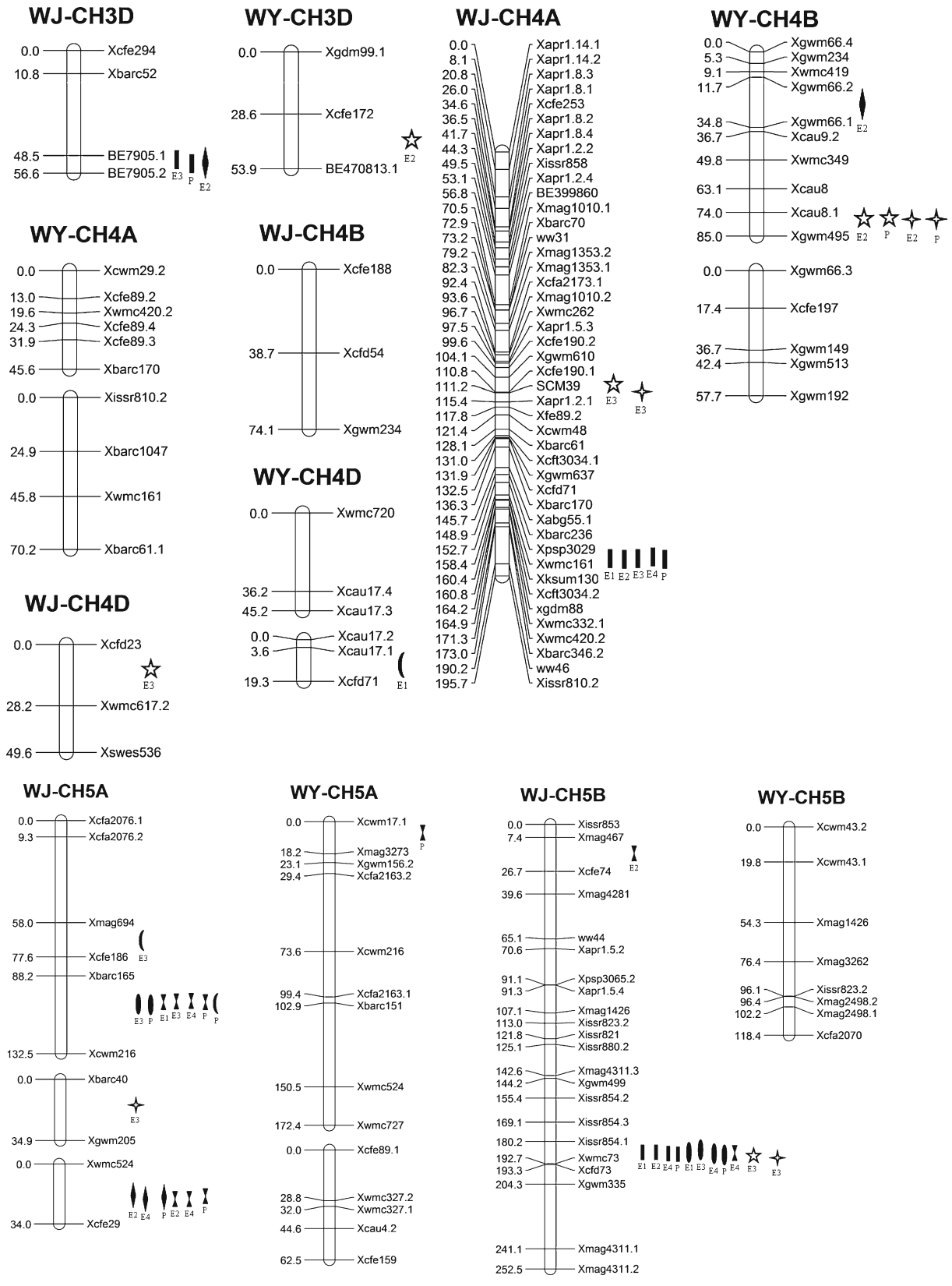

Figure 1 (*contd*)

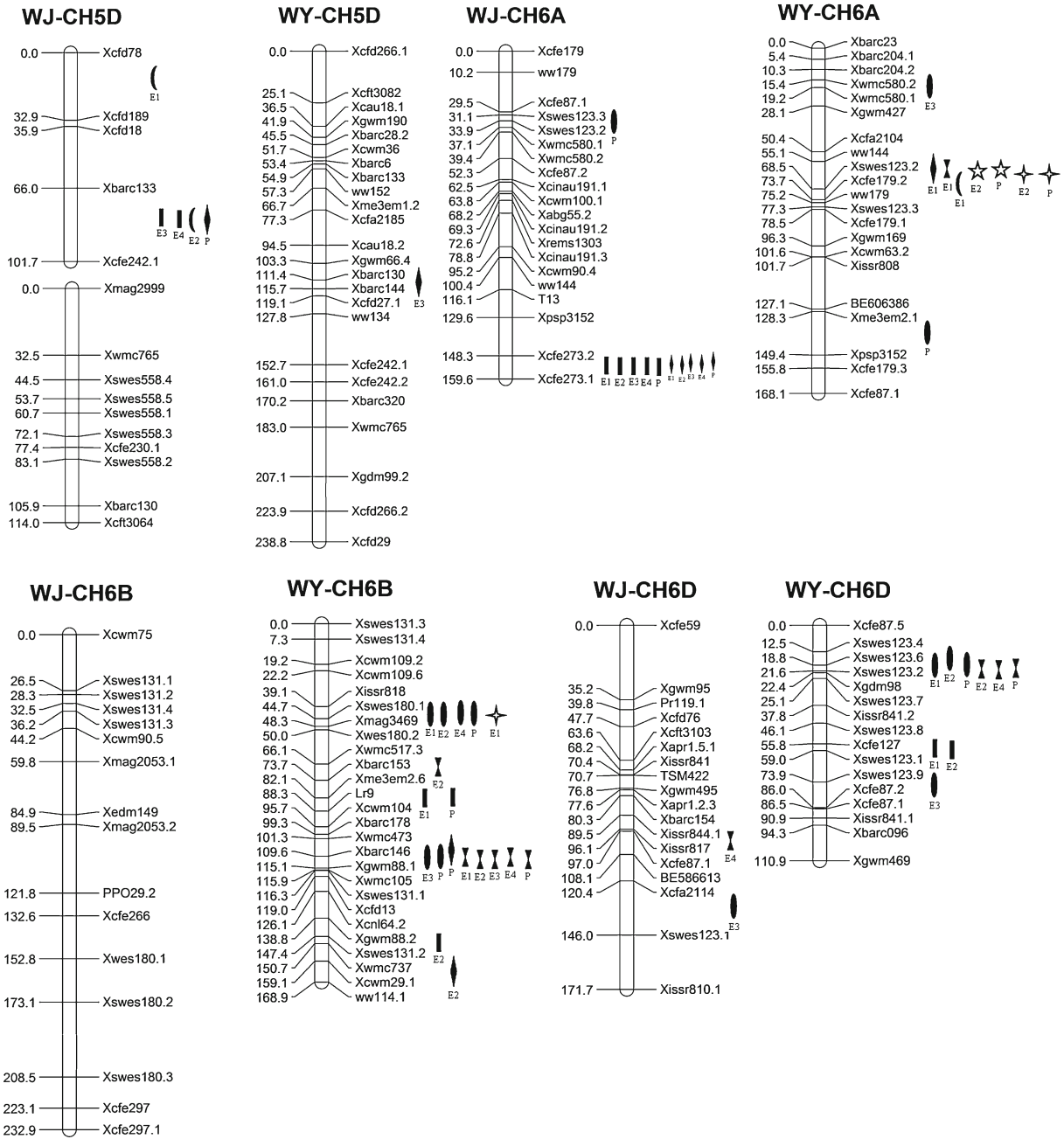

Figure 1 (*contd*)

meaning that this QTL for TKW is entirely contributed by KL; (iv) an additional QTL can be detected by the conditional mapping method, which means that the expression of the QTL for TKW is completely suppressed by KL, and the effects could only be identified by eliminating the influence of KL. This suggests that the additional QTL has an opposite additive effect on KL and the other causal trait of TKW.

The present study, together in the two populations, indicated that only two QTL, *QTkw-WJ-1A.1* and *QTkw-WJ-5D-2.2*, were both entirely due to variation in all the three TKW-related traits referred here, i.e., KL, KW and KDR (tables 3 and 4). Three QTL, *QTkw-WJ-2D-1.1*, *QTkw-WJ-7A.2b* and *QTkw-WY-7D.1*, were completely contributed by both KL and KW; however, KDR contributed to them at different levels. Five QTL, *QTkw-WJ-1A.2*, *QTkw-WJ-2B.2*, *QTkw-WY-6B.1*, *QTkw-WY-6D.2* and *QTkw-WY-7B.1a*, were detected entirely due to variation in both KW and KDR; however, KL contributed to them at different levels, and sometimes they showed inconsistency across environments. Four QTL, *QTkw-WJ-3B.3*, *QTkw-WJ-7A.3*, *QTkw-WY-6B.2* and *QTkw-WY-7B.1b*, were all entirely contributed by KW consistently over trials in which they were detected; KL made no

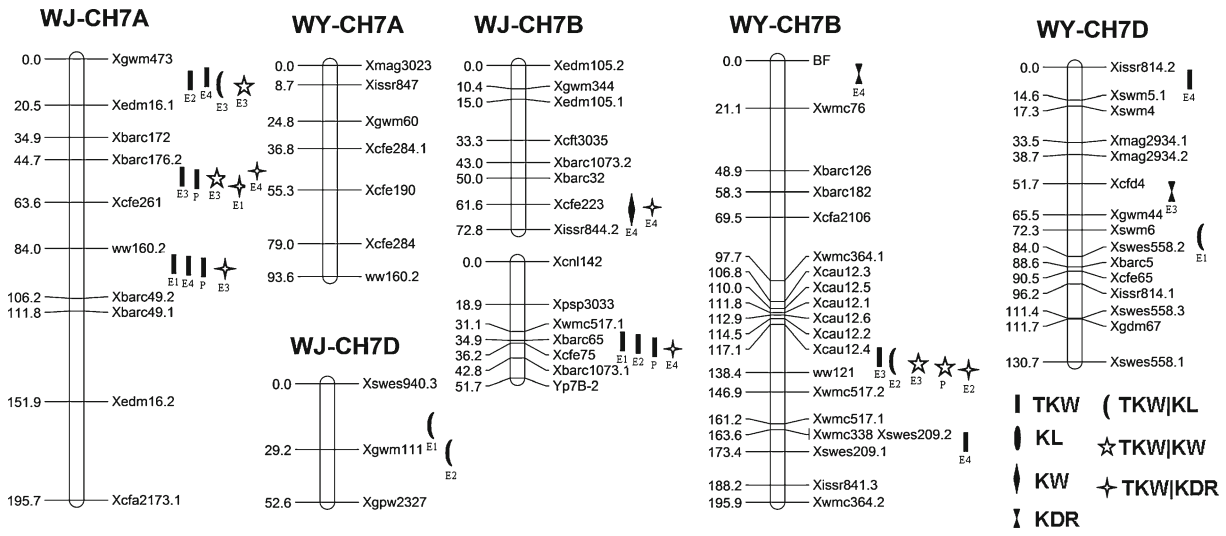

Figure 1 (*contd*)

contribution except for one trial where they were detected, but made entire contribution to them in the remaining trials, showing inconsistency across environments. Both *QTkw-WJ-7B-2.3* and *QTkw-WY-2B-1.3* expressed entirely dependent of variation in KL, but independent of variation in KDR, in all trials where they showed significance; however, KW entirely contributed to them in one trial each and partially contributed to them in the remaining two trials each. For three QTL, *QTkw-WJ-4A.5*, *QTkw-WJ-6A.5* and *QTkw-WJ-7A.2a*, KDR did not have any impact on their expression in all trials where they showed significance; KL and KW had inconsistent influence on these QTL over trials. *QTkw-WJ-5B.4* was entirely/partially due to variation in KL, but KW had no influence on it; due to strong association between KDR and KL, this QTL was partially contributed by KDR consistently over trials. *QTkw-WJ-3D.2* was partially due to variation in KW, KL; but KDR had inconsistent influences on it over trials. *QTkw-WY-2A.1* was completely contributed by KDR, partially contributed by KL, but KW made no contribution to it. For *QTkw-WY-2A.3*, all the three TKW-related traits referred here had inconsistent influences on it.

In addition, many QTL for TKW were undetectable in unconditional QTL mapping analysis due to the interference of related traits (tables 5 and 6; figure 1). Of the extra conditional QTL for TKW, some were suppressed simultaneously by two or three related traits referred here (figure 1). Of these, five each were detected when TKW were conditioned on KW and KDR, respectively, and they were pairwise co-located on 3B, 4A, 5B and 7A in WJ, and 4B in WY, respectively (tables 5 and 6; figure 1). Conditioning TKW on KL and KW, respectively, one each extra conditional QTL for TKW was detected on chromosome 7B in WJ, and they shared a common interval, as did one pair of conditional QTL on 2A in WY, which was identified when the influence of KL and KDR on TKW were excluded, respectively. Two conditional QTL for TKW in WY were both suppressed by all the three TKW-related traits, distributing on chromosome 6A and 7B, respectively. Notably, several unconditional QTL were detected in conditional QTL mapping analysis in the trial in which they failed to show significance in unconditional QTL mapping analysis. Though we considered them as extra conditional QTL for TKW, they shared common intervals with that of their corresponding unconditional QTL.

The results described above demonstrate that some unconditional QTL for TKW were contributed by more than one component trait, based on whether the QTL could be identified by conditional analysis (tables 3 and 4). In QTL mapping, the likelihood of detecting a QTL is dependent on the ratio between the variance caused by the QTL's effect and the total variance of the trait, as well as the size of the mapping population (Lander and Botstein 1989). In conditional QTL analysis, effects on QTL contributed by a conditional trait are reduced and the QTL with effects below a certain threshold become virtually undetectable. Thus, it is reasonable to obtain the results described, which indicated that the unconditional QTL for TKW was strongly influenced by the conditional traits, indicating a pleiotropic QTL. Notably, there was a difference in the conditional mapping results in different environments. The environment plays an important role in controlling gene expression, especially for quantitative traits, and could account for the above differences.

Conditional QTL mapping analysis above indicated} that, at the QTL level, KW had the strongest influence on} TKW, next to KL, and KDR had the least level of contribution} to TKW (tables 3, 4, 5 and 6). This finding confirmed the} results of the correlation analysis and variance analysis in table} 2, and is also consistent with previous researches (Dholakia *et al.* 2003; Sun *et al.* 2009; Gegas *et al.* 2010). Therefore, to increase TKW, we should enhance KW, in accordance with practical wheat breeding.

Table 3. Conditional QTL for thousand-kernel weight with respect to kernel dimensions in the WJ population.

Uncon[a]	Condition add (En/PVE%)[b]		
	TKW|KL	TKW|KW	TKW|KDR
QTkw-WJ-1A.2	0.83 (E3/3.51) −		
QTkw-WJ-1A.1			
QTkw-WJ-2B.2	0.80 (E2/4.06) =		
QTkw-WJ-2D-1.1			−0.77 (E2/2.21) −
QTkw-WJ-3B.3	1.12 (E1/4.88) =		0.95 (E1/3.46) −
			0.83 (E2/3.11) +
QTkw-WJ-3D.2	−0.94 (E3/5.25) +	−0.79 (E3/4.72) +	−0.71 (E3/2.77) =
	−0.67 (P/2.99) =	−0.47 (P/2.42) −	
QTkw-WJ-4A.5	0.87 (E1/3.03) −	0.68 (E1/3.20) −	1.23 (E1/6.04) =
		1.03 (E2/7.11) +	0.81 (E2/3.00) =
			0.75 (E3/5.48) =
			0.87 (E4/3.97) =
			0.86 (P/4.39) =
QTkw-WJ-5B.4	−0.72 (E3/2.96) −	−0.99 (E1/4.49) =	−1.25 (E1/4.40) +
	−0.64 (P/2.78) −	−1.15 (E2/8.16) =	−1.30 (E2/6.71) +
		−1.11 (E3/8.70) =	−1.26 (E3/7.19) +
		−0.96 (P/9.82) =	−1.23 (P/4.92) +
QTkw-WJ-5D-2.2			
QTkw-WJ-6A.5	−1.13 (E1/5.14) −	−0.83 (E2/4.82) −	−1.22 (E1/4.57) =
	−1.16 (E2/7.54) =		−1.04 (E2/4.90) =
	−0.81 (E4/4.33) −		−0.69 (E3/2.83) =
	−0.79 (P/4.70) −		−1.09 (E4/6.12) =
			−0.92 (P/4.92) =
QTkw-WJ-7A.2a	−1.06 (E4/6.83) +	−0.86 (E4/5.41) =	−0.96 (E2/4.16) =
			−1.02 (E4/6.12) =
QTkw-WJ-7A.2b			−0.76 (P/3.30) −
QTkw-WJ-7A.3	0.78 (E1/2.34) =		0.91 (E4/4.16) +
			0.58 (P/1.92) −
QTkw-WJ-7B-2.3		1.54 (E1/5.89) −	1.88 (E1/4.89) =
		0.84 (P/3.59) −	1.41 (E2/3.66) =
			1.15 (P/2.92) =

Here, we only compared the conditional QTL and unconditional QTL in the trials where the unconditional QTL showed significance. [a]Unconditional QTL. [b]Conditional QTL. TKW|KL, TKW without the influence of KL; TKW|KW, TKW without the influence of KW; TKW|KDR, TKW without the influence of KDR. Numerals before parentheses are estimates of the additive effect of the conditional QTL. A letter E plus a numeral, or the letter P, in parentheses, indicate the environment in which the conditional QTL was detected. The numeral after the slash in parentheses is the percentage of phenotypic variance explained by the additive effects of the mapped QTL. A minus, '−', or a plus, '+', following the parentheses, denotes the additive effect of a conditional QTL, in absolute, that increases or decreases more than 10% of that of the corresponding unconditional QTL, respectively. An equal sign, '=', was placed after the parentheses if a conditional QTL with equal additive effects to that of the unconditional QTL. An unconditional QTL that still showed significance in conditional analysis in a trial in which it did not show significance in unconditional analysis is given in bold font. For the remaining abbreviations and descriptions see table 1.

Determining genetic relationship of a pairwise QTL for two related/causal traits based on their co-location: are the results consistent with that of conditional QTL mapping analysis?

Coincidence of QTL may indicate either single QTL with pleiotropic effects or that the genomic regions associated with these QTL harbour a cluster of linked genes associated with those traits. Generally, QTL for pairwise traits that have strong genetic association are prone to be co-located. However, unconditional QTL for a complex trait are usually under the interference of its more than one causal traits, thus we can hardly clarify the actual relationships between the complex trait and its certain causal trait at an individual QTL level through unconditional analysis (Wen and Zhu 2005; Ye *et al.* 2009).

Indeed, the present study confirmed this theory, as the co-location of QTL for pairwise traits was not always consistent with that of conditional QTL mapping analysis (figure 1). Based on the conditional QTL mapping analysis, we knew that all the unconditional QTL for TKW detected in the two populations were either completely or partially contributed by at least one of the three TKW-related traits referred here. This implied that all the unconditional QTL for TKW should be co-located with at least one QTL for KW, KL, or KDR; however, six unconditional QTL for TKW in WJ and eight in WY did not show pleiotropic effects; in other words, no QTL

Table 4. Conditional QTL for thousand-kernel weight with respect to kernel dimensions in the WY population.

Uncon[a]	Condition add (En/PVE%)[b]		
	TKW|KL	TKW|KW	TKW|KDR
QTkw-WY-2A.1	−1.56 (E1/10.94) +	−1.39 (E1/12.23) =	
QTkw-WY-2A.3	0.98 (E3/6.79) −	0.78 (E3/5.67)−	1.13 (E3/8.40) =
	−1.92 (E4/16.71) +	−1.93 (E4/20.15) +	1.55 (E4/11.18) =
QTkw-WY-2B-1.3		−1.29 (E3/15.34)−	−1.45 (E1/9.03) =
		−0.97 (P/12.74) −	−1.35 (E3/14.79) =
			−1.29 (P/11.06) =
QTkw-WY-6B.2	−1.29 (E1/6.95) =		−1.50 (E1/9.51) =
QTkw-WY-6B.1	1.04 (E2/8.49) =		
QTkw-WY-6D.2	−1.11 (E1/5.71) =		
QTkw-WY-7B.1a	−0.99 (E3/6.89) −		
QTkw-WY-7B.1b	−1.15 (E4/8.01) =		−1.17 (E4/5.57) =
QTkw-WY-7D.1			−1.07 (E4/4.91) =

See table 3 for title descriptions.

for KL, KW or KDR was co-located with them. Of these, in WJ, one each was distributed on chromosomes 1A, 4A and 7B, respectively, and three on 7A; in WY, one each was located on chromosomes 6D and 7D, two each on 2A, 6B and 7B, respectively (figure 1). Though the remaining nine QTL co-segregated with one or more QTL for its related traits,

Table 5. Extra conditional QTL for thousand-kernel weight with respect to kernel dimensions in the WJ population.

Trait[a]	Extra conditional QTL[b]	Interval[c]	En.[d]	LOD[e]	PVE%[f]	Add[g]
TKW|KL	*QTkw|kl-WJ-2A.1*	*Xgwm382.3–Xgwm558*	P	4.10	4.87	0.85
	QTkw|kl-WJ-2B.2	*Xbarc98–Xwmc332.2*	E4/P	3.61/3.06	5.77/4.42	1.18/0.97
	QTkw|kl-WJ-2D-2.1	*PPO29.1–Xmag633*	P	2.76	3.19	0.66
	QTkw|kl-WJ-3A.2	*Xswes185–Xmag896.1*	E3/P	3.88/5.24	3.76/4.77	−0.76/−0.81
	QTkw|kl-WJ-5A-1.1a	*Xmag694–Xcfe186*	E3	4.78	8.39	−1.41
	QTkw|kl-WJ-5A-1.1b	*Xbarc165–Xcwm216*	P	3.32	11.09	−1.29
	QTkw|kl-WJ-5D-1.1a	*Xcfd78–Xcfd189*	E1	3.19	2.53	−0.84
	QTkw|kl-WJ-5D-1.1b	*Xbarc133–Xcfe242.1*	**E2 (E3/E4)**	3.35	4.62	−0.91
	QTkw|kl-WJ-7A.1	*Xgwm473–Xedm16.1*	**E3 (E2/E4)**	3.34	4.48	−0.86
	QTkw|kl-WJ-7D.2	*Xswe2940.3–Xgwm111*	E1/E2	3.05/3.14	3.56/3.45	−0.96/−0.85
TKW|KW	*QTkw|kw-WJ-3B.1*	*Xbarc164–Xcft53*	E4	2.9	3.36	0.66
	QTkw|kw-WJ-3B.2	*Xbarc229.2–Xcwm93*	E1/P	3.20/2.81	5.15/4.01	0.86/0.57
	QTkw|kw-WJ-4A.1	*Xcfe190.1–Xapr1.2.1*	E3	3.73	3.70	−0.70
	QTkw|kw-WJ-4D.1	*Xcfd23–Xwmc617.2*	E3	3.87	3.92	0.70
	QTkw|kw-WJ-5B.1	*Xissr854.1–Xwmc73*	**E3 (E1/E2/E4/P)**	2.57	2.21	−0.55
	QTkw|kw-WJ-7A.1a	*Xgwm473–Xedm16.1*	**E3 (E2/E4)**	3.09	3.69	−0.68
	QTkw|kw-WJ-7A.1b	*Xbarc176.2–Xcfe261*	**E1 (E3/P)**	3.12	2.92	−0.68
TKW|KDR	*QTkw|kdr-WJ-1D-2.1*	*Glu-d1–Xbarc346.1*	E1	5.88	6.84	1.68
	QTkw|kdr-WJ-2A.1	*Xdupw210–Xgwm382.1*	E3	4.05	3.70	−0.83
	QTkw|kdr-WJ-3B.1	*Xbarc164–Xcft53*	P	2.70	2.25	0.62
	QTkw|kdr-WJ-4A.1	*Xcfe190.1–Xapr1.2.1*	E3	4.76	5.80	1.00
	QTkw|kdr-WJ-5A-2.1	*Xbarc40–Xgwm205*	E3	3.39	6.82	−1.07
	QTkw|kdr-WJ-5B.1	*Xissr854.1–Xwmc73*	**E3 (E1/E2/E4/P)**	3.72	3.60	−0.83
	QTkw|kdr-WJ-7A.2	*Xbarc176.2–Xcfe261*	**E1/E4 (E3/P)**	3.24/2.63	3.68/3.65	−1.01/−0.85
	QTkw|kdr-WJ-7A.1	*ww160.2–Xbarc49.2*	**E3 (E1/E4/P)**	7.60	6.41	1.05
	QTkw|kdr-WJ-7B-1.1	*Xcfe233–Xissr844.2*	E4	2.62	4.02	0.97
	QTkw|kdr-WJ-7B-2.1	*Xbarc65–Xcfe75*	**E4 (E1/E2/P)**	3.01	2.82	1.21

[a]See table 3 for details. [b]A conditional QTL that still showed significance in unconditional analysis but in other trial/trials is marked by bold typeface. [c]Flanking markers of the QTL. [d]A letter E plus a numeral, or a letter P, before the parentheses, indicates a trial in which the conditional QTL showed significance in conditional analysis; and that in the parentheses indicates a trial in which the conditional QTL showed significance in unconditional analysis. In all trials where QTL showed significance in both conditional and unconditional analysis are given in bold font. [e]LOD value of the corresponding putative additive QTL. [f]Phenotypic variance explained by the corresponding putative additive QTL. [g]Additive effect of the corresponding putative additive QTL; positive values indicate Weimai 8 alleles that increase the value of the corresponding trait, and conversely, negative values indicate Weimai 8 alleles decrease it.

Table 6. Extra conditional QTL for thousand-kernel weight with respect to kernel dimensions in the WY population.

Trait[a]	Extra conditional QTL[b]	Interval[c]	En.[d]	LOD[e]	PVE%[f]	Add[g]
TKW|KL	***QTkw|kl-WY-2A.1***	*Xissr848–Xgwm372*	**P (E1)**	5.06	9.36	−1.03
	QTkw|kl-WY-4D-2.1	*Xcau17.1–Xcfd71*	E1	2.70	4.10	1.00
	QTkw|kl-WY-6A.1	*Xcfe179.2–Xswes123.3*	E1	3.22	6.61	1.21
	QTkw|kl-WY-7B.1	*Xcau12.4–ww121*	**E2 (E3)**	2.97	5.73	−0.88
	QTkw|kl-WY-7D.1	*Xswm6–Xswes558.2*	E1	2.74	4.11	−1.02
TKW|KW	*QTkw|kw-WY-1A.1a*	*Glu-a1–Xgdm93*	E2	3.06	9.44	1.13
	QTkw|kw-WY-1A.1b	*Xmag3489–Xme3em2.8*	E4	2.67	7.46	1.17
	QTkw|kw-WY-2A.2	*Xcfe175.2–Xcfe253.2*	E1/P	2.52/5.48	9.93/13.92	−1.31/−1.06
	QTkw|kw-WY-2B-1.1	*Xmag1729.1–Xgwm374*	P	2.88	8.19	−0.93
	QTkw|kw-WY-3D.1	*Xcfe172–BE470813.1*	E2	2.53	4.38	−0.71
	QTkw|kw-WY-4B-1.2	*Xcau8.1–Xgwm495*	E2/P	4.35/4.08	9.19/7.52	1.34/1.00
	QTkw|kw-WY-6A.2	*Xcfe179.2–Xswes123.3*	E2/P	4.67/3.14	9.62/6.11	−1.04/−0.67
	QTkw|kw-WY-7B.2	*Xcau12.4–ww121*	**E2/P (E3)**	3.31/2.60	7.49/5.26	−0.92/−0.63
TKW|KDR	***QTkw|kdr-WY-2A.1***	*Xissr848–Xgwm372*	**P (E1)**	4.85	18.15	−1.52
	QTkw|kdr-WY-2B-1.1	*Xcinau119.2–Xcinau119.1*	**E2 (E1/E3/P)**	2.61	8.70	−1.11
	QTkw|kdr-WY-4B-1.2	*Xcau8.1–Xgwm495*	E2/P	3.39/3.78	7.35/7.16	1.34/1.27
	QTkw|kdr-WY-6A.2	*Xcfe179.2–Xswes123.3*	E2/P	5.19/4.95	11.66/9.40	−1.28/−1.11
	QTkw|kdr-WY-6B.1	*Xswes180.1–Xmag3469*	E1	5.13	10.82	−1.74
	QTkw|kdr-WY-7B.1	*Xcau12.4–ww121*	**E2 (E3)**	2.67	4.77	−0.82

See table 5 for title descriptions.

they all accounted only for a part of the conditional QTL mapping analysis. For example, conditional analysis indicated that *QTkw-WJ-1A.2* was partially/entirely contributed by KL and entirely contributed by both KW and KDR; however, only *QKw-WJ-1A.2* co-located with it (table 3; figure 1).

Based on the above comparisons of results of conditional QTL mapping analysis and traditional analysis, we conclude that it will not precise or efficient enough to determine genetic relationships of two related/causal traits according to the co-locations of QTL for the pairwise traits. Conditional QTL mapping methods for multivariate conditional analysis is an efficient tool to reveal relationships between a complex trait and its causal traits.

QTL consistency over environments

If a QTL is independent of the environment, the implication is that its expression is stable regardless of differences in environment. We defined a stable QTL that was verified in at least three of the five trials. Together in WJ and WY populations, 24 QTL were stable and were involved in all the four traits referred here (table 7). Of these, *QKdr-WJ-5A-1.4*, *QTkw-WY-2A.3*, *QTkw-WY-2B-1.3*, *QKl-WY-6B.4* and *QKdr-WY-6B.5* were major QTL that accounted for more than 10% of the phenotypic variance with LOD scores of >3.0 (see tables 1 and 2 in electronic supplementary material). Generally, a major QTL consistent over environments is of great value for marker assisted selection (MAS) in breeding programmes; thus, the five major stable QTL should be of great value in genetic improvement of wheat kernel-related traits.

Two-related RIL populations with large/moderate size

If a QTL detection is conducted based on a population with limited lines, the number of QTL that can be detected are usually underestimated and their effects are prone to be overestimates (Beavis 1998; Bernardo 2004; Schön *et al.* 2004; Vales *et al.* 2005; Buckler *et al.* 2009). False positive QTL

Table 7. The number of unconditional QTL detected in the WJ and WY populations.

	No. of QTL								
	PVE%			No. of trial					
Trait	<5%	5%–10%	>10%	1	2	3	4	5	Total
TKW	9/0	5/6	0/3	2/5	6/2	3/2	1/0	2/0	14/9
KL	7/2	4/3	1/2	6/4	2/1	2/1	1/1	1/0	12/7
KW	9/1	4/4	0/4	9/8	2/1	1/0	1/0	0/0	13/9
KDR	7/2	4/9	1/2	5/9	1/2	4/1	2/0	0/1	12/13

For each entry, the first numeral refers to WJ, and the second, to WY. See table 1 for abbreviations and title descriptions.

Table 8. Congruent QTL resolved in both populations.

WJ[a]	WY[b]	Alleles[c]	Common loci[d]
QKw-WJ-1A.2	*QKw-WY-1A.1b*	+/−	*Glu-a1/Glu-a1*
QKdr-WJ-2A.4	*QKdr-WY-2A.2*	−/−	*Xbarc212/Xbarc212*
QKl-WJ-6A.1a	*QKl-WY-6A.1a*	+/−	*Xwmc580.1/Xwmc580.1*
QKl-WJ-6D.1	*QKl-WY-6D.1*	−/−	*Xswes123.1/Xswes123.1*
QTkw-WJ-7B-2.3	*QTkw-WY-7B.1b*	+/−	*Xwmv517.1/Xwmc517.1*

[a]QTL detected in the WJ population. [b]QTL detected in the WY population. [c]Additive effect; for additional details, see table 5. [d]Loci nearby the corresponding putative additive QTL are common in the two populations. [c, d]For each entry, the first signal refers to WJ, and the second, to WY.

might be caused by parental sharing when the RIL population was not large enough to permit completely random mating (Zou *et al.* 2005). Of the 51 unconditional QTL for the four kernel-related traits reported here, only two QTL, individually, explained more than 10% of the phenotypic variance (table 7). In 175 lines randomly sampled from the 485 RIL lines of WJ and a high-density genetic map enriched with DArT markers, however, 40 of the 57 unconditional QTL for the four kernel-related traits, individually, accounted for more than 10% of the phenotypic variance. Thirteen QTL showed significance simultaneously in analysis based on 485 and 175 lines of WJ RIL population, and each of them had much higher additive effects and contribution rates in 175 lines of WJ than 485 lines. For example, *QTkw-WJ-5B.4* explained 3.35, 4.22, 4.75 and 4.0% of the phenotypic variance with additive effect values of −1.09, −1.09, −1.03 and −0.91 g in E1, E2, E4 and P, respectively, in the 485 RIL lines of WJ; however, in the 175 lines of WJ RIL population, it showed significance in E2, E4 and P with additive effect values of −1.38, −1.61 and −1.71 g, accounting for 8.42, 12.59 and 15.45% of the phenotypic variance (data not shown). In the WY population, 11 of 38 unconditional QTL for the four kernel-related traits accounted for more than 10% of the phenotypic variance, individually (table 7). QTL analysis based on 172 lines randomly sampled from the 229 RIL lines of WY and the high-density genetic map enriched with DArT markers, 45 unconditional QTL for the four kernel-related traits were identified, 33 of which individually explained more than 10% of the phenotypic variance. Fifteen QTL were significant in analysis based on both 172 and 229 lines of the WY RIL population, with equal or slight higher additive effects and contribution rates in the 172 lines of WY compared to that in the 229 lines of WY (data not shown). The above analysis indicates that: (i) it is difficult to detect minor QTL using a small population; (ii) QTL effects are apt to be overestimated with small populations; and (iii) the coincidence of QTL across environments may be influenced by the population size to some extent.

In addition, if we conducted QTL detection using a single mapping population, only a limited number of QTL could be detected and the result was not conclusive. With the rapid development of molecular marker technology, additional research on QTL effects in more than one different or related genetic backgrounds is warranted (Kumar *et al.* 2007; Ma *et al.* 2007; Breseghello and Sorrells 2007; Buckler *et al.* 2009; Uga *et al.* 2010; Gegas *et al.* 2010). Breseghello and Sorrells (2007) have shown that QTL detected on different mapping populations, with identical evaluation methods, can be very distinct. However, common QTL among related/associated mapping populations have been proven to be detectable when the methods of evaluation are identical (Ma *et al.* 2007; Buckler *et al.* 2009). Present study detected at least five pairwise congruent QTL in the two related RIL populations, based on common markers in the two genetic maps (table 8; figure 1). Of these, the common parent Weimai 8 alleles of two congruent QTL showed consistent additive effects, being positive simultaneously in the two populations. As is well known, a reducing or enhancing additive effect is not absolute but relative to the effect of two parental alleles, so the remaining three QTL can still be regarded as congruent QTL. Due to the limited number of common loci in the two genetic maps, the precise prediction and definition of common QTL in the two populations were hampered, although positions of most QTL for the same trait identified in the two populations were of high congruency. The results showed that QTL from the common parent in the two related populations can be detected repeatedly to a certain extent, and the comparable QTL are authentic.

Comparison of the present study with previous studies

TKW, KL and KW in wheat has been subjected to monosomic or QTL analysis in many other reports. In most cases, they were reported individually but not simultaneously in one report (Halloran 1976; Giura and Saulescu 1996; Shah *et al.* 1999; Araki *et al.* 1999; Kato *et al.* 2000; Ammiraju *et al.* 2001; Varshney *et al.* 2000; Zanetti *et al.* 2001; Böner *et al.* 2002; Huang *et al.* 2003, 2004, 2006; Groos *et al.* 2003; Campbell *et al.* 2003; McCartney *et al.* 2005; Verma *et al.* 2005; Li *et al.* 2007; Kirigwi *et al.* 2007; Röder *et al.* 2008; Hai *et al.* 2008; Golabadi *et al.* 2010; McIntyre *et al.* 2010; Su *et al.* 2010; Zheng *et al.* 2010). Though several other reports have documented QTL for TKW and KD simultaneously by traditional QTL mapping analysis or association mapping analysis, no conditional QTL mapping method was implemented to dissect their genetic relationships (Giura and

Saulescu 1996; Campbell *et al.* 1999; Dholakia *et al.* 2003; Breseghello and Sorrells 2006, 2007; Sun *et al.* 2009; Ramya *et al.* 2010; Tsilo *et al.* 2010). The present study first evaluated the genetic relationship between KW and KD at the level of an individual QTL using unconditional and conditional QTL mapping methods, thus enhancing the understanding of genetic control system involved in the synthetic process of TKW and KD. In addition, the combination of two related populations with a large/moderate population size made the results authentic and accurate.

Most QTL reported here are consistent with the previous reports. *QTkw-WJ-1A.2* and *Qkw-WJ-1A.2*, a pairwise pleiotropic QTL, correspond to a pairwise pleiotropic QTL for TKW and KW reported by Campbell *et al.* (1999); in addition, Varshney *et al.* (2000) and Ramya *et al.* (2010) have detcted QTL for TKW in this interval; this chromosomal region may harbour a robust QTL cluster for kernel-related traits, hence being potentially more useful in breeding programmes. *QKl-WJ-2D-2.3* and *QKdr-WJ-2D-2.3*, another pairwise pleiotropic QTL, are consistent with the co-loctaed QTL for KW and TKW reported by Ramya *et al.* (2010); also, Wang *et al.* (2009) have located a QTL for TKW in this interval; using 175 lines randomly sampled from the 485 RIL lines of WJ and the high-density genetic map enriched with DArT markers, these two QTL have been reproducibly detected, accounting for 9.88–16.16% and 5.58–15.18% of the the phenotypic variance, respectively (data not shown); thus, more attention should be paid to this chromosomal region in MAS in wheat breeding programmes. *QTkw-WJ-4A.5*, a environment-independent QTL, is in agreement with the QTL for KL revealed by Sun *et al.* (2009). *QTkw-WJ-5B.4*, *QKl-WJ-5B.4* and *QKdr-WJ-5B.1b* shared a common interval on chromosome 5B; in addition, when TKW was conditioned on KDR and KW, *QTkw|kdr-WJ-5B.1* and *QTkw|kw-WJ-5B.1* showed significance in a new environment in this interval; Ramya *et al.* (2010) have reported a pleiotropic QTL for TKW and KW in this interval; as *QKl-WJ-2D-2.3* and *QKdr-WJ-2D-2.3*, *QTkw-WJ-5B.4* showed significance in the small WJ RIL population, with additive effect values of −1.38 to −1.71 g, accounting for 8.42–15.45% of the phenotypic variance (data not shown); hence, this interval may be potentially of great value in breeding programmes. Both *QKl-WJ-5A-1.2* and *QKdr-WJ-5A-1.4* were mapped to a position similar to that of *Vrn1*, corresponding to the QTL for TKW detected by Campbell *et al.* (1999) and Wang *et al.* (2009); in addition, when TKW was conditioned on KL, *QTkw|kl-WJ-5A-1.1b* showed significance in this interval. The positions of *QTkw-WJ-7A.3* and a QTL for TKW reported by Huang *et al.* (2003) are of high congruency. *QTkw-WJ-7B-2.3* and *QTkw-WY-7B.1b*, a pairwise congruent QTL, were detected in WJ and WY populations, respectively; Hai *et al.* (2008) have reported a QTL for TKW in this interval, indicating a reliable QTL; in addition, the *Vrn-B3* gene was about 3.0 cM distal from *Xbarc65*, one flanking marker of *QTkw-WJ-7B-2.3*, indicating pleiotropic effects (http://wheat.pw.usda.gov/GG2/index.shtml). *QKl-WJ-3B.5* and *QKdr-WJ-3B.3* showed pleiotropic effects; the two QTL are of high congruency in position to the QTL for TKW detected Huang *et al.* (2006), Wang *et al.* (2009) and Golabadi *et al.* (2010). *QTkw|kw-WY-4B-1.2* and *QTkw|kdr-WY-4B-1.2*, two extra conditional QTL for TKW without the influences of KW and KDR, respectively, shared intervals of QTL for TKW reported by Huang *et al.* (2004, 2006), McCartney *et al.* (2005), Zheng *et al.* (2010), and for KL reported by Sun *et al.* (2009); in addition, *Rht-B1* was about 3.0 cM distal from *Xgwm495*, one flanking marker of the two extra conditional QTL (http://wheat.pw.usda.gov/GG2/index.shtml); this chromosomal segment should be a gene-rich region. One extra conditional QTL for TKW excluding the influences of KL, *QTkw|kw-WY-4D-2.1*, was mapped to a position similar to *Rht-D1*. *QKw-WJ-5A-3.3* and *QKdr-WJ-5A-3.3*, two co-located QTL, confirmed a QTL for KL and a QTL for TKW detected by Ramya *et al.* (2010) and Zheng *et al.* (2010), respectively. *QKdr-WJ-5A-1.4* and *QKl-WJ-5A-1.2* showed pleiotropic effects and they shared common intervals of QTL for TKW reported by Wang *et al.* (2009) and for kernel diameter reported by Tsilo *et al.* (2010). *QTkw-WY-2A.3* corresponds to a QTL for TKW reported by Huang *et al.* (2004); a QTL cluster for kernel weight, kernel diameter and kernel size has been reported in this interval by Tsilo *et al.* (2010); thus, this interval may be potentially of great value in breeding programmes. *QKdr-WY-6B.5*, *QKl-WY-6B.2* and *QKw-WY-6B.1a* are three co-located QTL; Huang *et al.* (2006) have detcted QTL for TKW in this interval. *QKl-WY-6D.3* and *QKdr-WY-6D.3*, two co-located QTL, have not been reported elsewhere, as has *QKl-WY-6B.4*. For the remaining QTL, comparison of the present study with previous studies was hampered due to lack of common information for their flanking markers of the corresponding QTL.

In summary, the combination of conditional and unconditional mapping methods applied to two related populations can precisely evaluate genetic relationship between KW and KD at an individual QTL level. In addition, a large population size can enhance the authenticity and accuracy of the QTL detection. Five major QTL that showed consistency in expression across environments will be of great value for MAS in breeding programmes.

Acknowledgements

This research was supported by the National Basic Research Programme of China (973 Programme, 2006CB101700). The authors thank Sishen Li, College of Agronomy, Shandong Agricultural University, Taian, China, for kindly providing EST-SSR markers.

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Received 6 January 2011, in final revised form 31 May 2011; accepted 2 June 2011
Published on the Web: 02 December 2011