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ORIGINAL PAPER

Mapping quantitative trait loci for quality factors in an inter-class cross of US and Chinese wheat

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Abstract Wheat quality factors are critical in determining the suitability of wheat (*Triticum aestivum* L.) for end-use product and economic value, and they are prime targets for marker-assisted selection. Objectives of this study were to identify quantitative trait loci (QTLs) that ultimately influence wheat market class and milling quality. A population of 132 F_{12} recombinant inbred lines (RILs) was derived by single-seed descent from a cross between the Chinese hard wheat line Ning7840 and the soft wheat cultivar Clark and grown at three Oklahoma locations from 2001 to 2003. Milling factors such as test weight (volumetric grain weight, TW), kernel weight (KW), and kernel diameter (KD) and market class factors such as wheat grain protein content (GPC) and kernel hardness index (HI) were characterized on the basis of a genetic map constructed from 367 SSR and 241 AFLP markers covering all 21 chromosomes. Composite interval mapping identified eight QTLs for TW, seven for KW, six for KD, two each for GPC and HI measured by near-infrared reflectance (NIR) spectroscopy, and four for HI measured by single kernel characterization system. Positive phenotypic correlations were found among milling factors. Consistent co-localized QTLs were identified for TW, KW, and KD on the short arms of chromosomes 5A and 6A. A common QTL was identified for TW and KD on the long arm of chromosome 5A. A consistent major QTL for HI peaked at the *Pinb-D1* locus on the short arm of chromosome 5D and explained up to 85% of the phenotypic variation for hardness. We identified QTLs for GPC on 4B and the short arm of 3A chromosomes. The consistency of quality factor QTLs across environments reveals their potential for marker-assisted selection.

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Introduction

The economic value of wheat (*Triticum aestivum* L.) is framed by intrinsic quality factors that affect the end-use product (Ammiraju et al. 2001; Morris and Rose 1996). Physical factors, described by test weight (TW), kernel weight (KW), and kernel size, partially determine milling yield if not also agronomic yield (Dholakia et al. 2003; Varshney et al. 2000). Wheat class factors, described by kernel hardness and grain protein content (GPC), broadly define functionality of the grain (non-leavened vs. leavened products) as well as the type of milling process and the physical nature of the milled product (Bushuk 1998; Khan et al. 2000; Lillemo and Ringlund 2002).

As a result of genetic analysis with classical and aneuploid methods, several hundred wheat genes have been identified, but functions and effects have been described for only a few of these. Among these genes, qualitative differences in kernel hardness can be explained by allelic differences at two loci on the short arm of chromosome 5D, *Pina-D1* and *Pinb-D1*, which encode the lipid-binding proteins puroindoline a and puroindoline b, respectively. Kernel hardness levels appropriate for different types of wheat-based food applications are related to specific allelic differences at these loci (Martin et al. 2001; Wrigley et al. 2009). Though extensively studied, wheat GPC has proven to be one of the more difficult traits to genotype. To date, several quantitative trait loci (QTL) for GPC have been identified: *pro*1 and *pro*2 on chromosomes 5D and 5A and unnamed QTLs on 2D (Prasad et al. 1999; Huang et al. 2006), 4D (Groos et al. 2003; Huang et al. 2006), 6B (Distelfeld et al. 2004; Khan et al. 2000), 7B (Huang et al. 2006), and 2A, 3A, and 7D (Groos et al. 2003). More recently, a GPC gene on 6BS has been cloned (Uauy et al. 2006).

Earlier studies on physical factors reported that TW is influenced by kernel shape, uniformity, density, and packing efficiency (Campbell et al. 1999; Galande et al. 2001). Kernel weight and size are controlled by several QTLs with various effects on 15 different chromosomes (Campbell et al. 1999; Galande et al. 2001; Dholakia et al. 2003; Groos et al. 2003; Huang et al. 2006). Unfortunately, genetic improvement in KW may be compromised by a concomitant reduction in kernel number per spike, thus neutralizing the agronomic benefit derived from increased KW (Marshall et al. 1984; Wiersma et al. 2001). However, relatively small increases in KW or kernel size, at the same yield level, should have a proportionately favorable effect on milling quality.

Using a high-density AFLP and SSR map, our objectives were to identify QTLs affecting wheat quality factors in winter wheat by, estimate their magnitude, and determine their chromosomal locations.

Materials and methods

Genetic materials and experimental design

A population of 132 $F_{8:12}$ recombinant inbred lines (RILs) was derived by single-seed descent from the F_2 of the Ning7840/Clark cross (Bai et al. 1999). Ning7840 (Aurora/Anhui 11//Sumai 3) is a hard red facultative breeding line from China with type II resistance to wheat scab and relatively low yield potential in Oklahoma. Clark is a soft red winter (SRW) wheat cultivar from Purdue University, IN, with an early date of heading, relatively high yield potential, and high KW (Ohm et al. 1988). The quality traits of the RILs along with the parents were evaluated for seven combinations of years and locations at Stillwater (2001, 2003 and 2003), Lahoma (2002 and 2003), and Altus (2002 and 2003), Oklahoma, by using a replicates-in-sets design with three replications and a plot size of 1.4 m^2 planted at a density of 58 kg ha^{-1}.

Traits

Data for TW were collected in this mapping population from all seven environments. Data for other wheat quality factors were obtained from five environments, excluding the 2002 Lahoma and 2003 Altus environments. Test weight was measured from the weight of grain filling a 0.95 L container and converted to kg hL^{-1}. The single kernel characterization system (SKCS; Model 4100, Perten Instruments North America, Inc., Springfield, IL, USA) was used to estimate KW (mg), KD (mm), and hardness index (HI-SK, scale of 0 = extremely soft to 100 = extremely hard) from a sample of 300 sound kernels per plot. Wheat grain protein content (g kg^{-1}) and another assessment of HI (same 0–100 scale) were determined by near-infrared reflectance (NIR) spectroscopy, designated as HI hereafter, according to AACC method 39-70a (American Assoc. Cereal Chem, 1995) using 9 g of ground whole wheat samples from each plot. Trait measurements were taken from at least five environments (Supplemental Table 1).

Isolation and amplification of DNA

Genomic DNA from both parents and the 132 F_{12} RILs was extracted by the cetylmethylammonium bromide method (Bai et al. 1999). Parental polymorphism was assessed with 1,500 SSR primers, including BARC (Song et al. 2005), GWM (Röder et al. 1998), WMC (Somers et al. 2004), GDM (Pestsova et al. 2000), CFA and CFD (Guyomarc'h et al. 2002; Sourdille et al. 2003), and DUP (Eujayl et al. 2002). A total of 365 polymorphic markers were analyzed in the RILs. SSR PCR setup and amplification followed Liu et al. (2008). Amplified PCR fragments were separated by using an ABI Prism 3730 DNA Sequencer (Applied Biosystems, Foster City, CA, USA) (Liu et al. 2008). The SSR data were scored by using GeneMarker software version 1.6 (SoftGenetics LLC, State College, PA, USA). The two parents and the 132 RILs were previously characterized with AFLP markers (G. Bai, unpublished results), producing 618 polymorphic band readings according to the method described by Bai et al. (1999).

Linkage mapping

To construct a genetic linkage map, segregating SSR and AFLP markers were scored visually for each RIL and recorded as either type 'A' (Ning7840) or 'B' (Clark),

whereas ambiguous bands were scored as missing (−). Linkage analysis was performed using the JoinMap program version 3.0 (Van Ooijen and Voorrips 2001). Recombination frequencies were converted to centimorgans (cM) with the Kosambi mapping function (Kosambi 1944). The genetic map was initially constructed with all mappable SSR and AFLP markers and refined by removing overlapping or very closely linked AFLP markers.

Statistical analysis

Skewness and kurtosis were estimated from the phenotypic distribution of entry means to determine departure from normality. Data from each environment were subjected to analysis of variance (ANOVA) to determine random effects of genotype (RIL and parent) after removing the environmental effects of sets and replicates within sets (SAS Institute, Cary, NC, USA, version 9.1). Broad-sense heritability was calculated by the formula $H2 = V_g/(V_g + (V_{ge})/r + V_e/re)$, in which the respective variance components are attributed to genotypic, genotype environment and experimental error effects, r is the number of replicates per environment, and e is the number of environments for a given trait. Phenotypic correlations were calculated for all combinations of traits on the basis of RIL means across environments. Principal coordinate analysis (PCA) of genotypes across environments was performed using standardized ($\mu = 0$, $\sigma = 1$) means in the PRINCOMP procedure of SAS (SAS Institute, Cary, NC, USA, version 9.1). Briefly, the resulting principle coordinate (PC) scores for genotypes and traits were plotted in a biplot, and trait vectors were drawn from the origin to their corresponding coordinates. An angle formed between two trait vectors approximated their correlation, with 0° and 180° angles indicating strong correlations (positive and negative, respectively) and 90° angles representing a weak correlation (Yan and Kang 2003).

QTL analysis

QTL Cartographer V2.5 was used to perform composite interval mapping (CIM) on the basis of model 6 of the Zmapqtl procedure (Wang et al. 2004). The closest marker to each local LOD peak was used as a cofactor. The walking speed for scanning the genome was set at 1.0 cM. The LOD threshold for declaring a significant QTL was estimated from 1,000 permutations of the data. Additive effects of the detected QTL were estimated by the Zmapqtl procedure. The proportion of phenotypic variance explained by a QTL was estimated as the coefficient of determination (R^2). For each QTL, R^2 was determined based on the R^2 for the single marker that was the closest to the target QTL. The total R^2 that represents the phenotypic variation explained by the model was calculated through multiple linear regressions using the SAS REG procedure. If a QTL was significant in at least two environments, it was considered a consistent QTL.

Results

Linkage map

In total, 380 polymorphic SSR and 615 AFLP markers were used to construct the linkage map for the Ning7840/Clark population. However, about 300 overlapping or very closely linked AFLP markers were removed in the final map used for QTL mapping. The final map consisted of 365 SSR and 229 AFLP markers covering 60 linkage groups of at least two markers. Fifty-seven linkage groups contained at least two SSR markers that were previously assigned to a specific chromosome (data not shown). The new map covered all 21 chromosomes and spanned 2203 cM with an average interval of 3.7 cM. The number of markers in each chromosome varied from 9 on 4D to 48 on 3B, covering 31–194 cM in genetic distance, respectively. Therefore, the saturated map was ideal for a whole-genome QTL scan.

Phenotypic variation of quality traits in RILs and parents

Between the parents, Clark produced heavier (29.7 mg KW) and larger (2.26 mm KD) kernels than Ning7840 (26.3 mg KW and 2.14 mm KD) across environments ($P < 0.05$). As expected for a SRW genotype, Clark produced lower values for both measurements of HI. Despite these differences in kernel size and texture, both parents had similar values for TW and GPC.

Most values for skewness and kurtosis did not exceed 1.0 (Supplemental Table 2), indicating the RIL phenotypic distributions exhibited normality except for HI (Fig. 1). The RILs segregated for a few genes with major effects on hardness, as indicated by the bimodal distributions for HI measured with both NIR and SKCS. That transgressive segregation occurred in both directions for all traits implies that both parents might contribute QTL with positive effects to these traits in this population. In general, all traits except kernel hardness exhibited continuous variation and polygenic segregation patterns.

Significant positive correlations ($P < 0.01$) were observed between TW and either KW or KD (Table 2), suggesting RILs with a higher TW tended to have heavier or larger kernels. Kernel weight and KD were also moderately associated with GPC. The PC biplot was used to reflect multi-trait relationships within the inference space

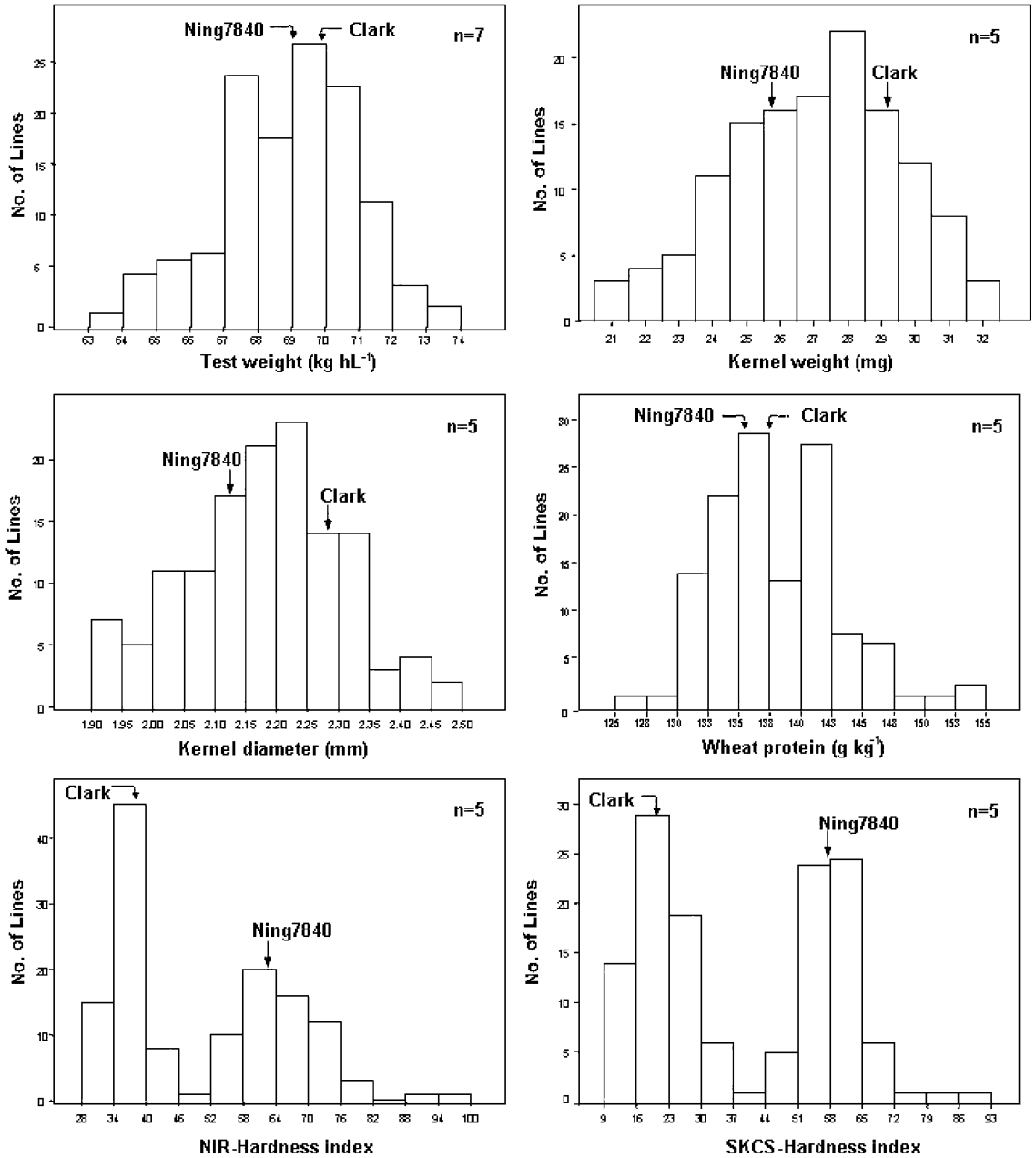

Fig. 1 Frequency distributions for wheat quality traits of 132 RILs averaged across *n* environments. Parental means of Ning7840 and Clark are indicated by *arrows*

of RIL variation (Fig. 2). Kernel size factors (KD and KW) were strongly associated with PC1, and two distinctive clusters of genotypes were formed along the PC2 axis according to HI. Kernel diameter and KW showed a strong association in the biplot, as did TW and KD. Protein content showed a close association with KW, but the relatively short vector for GPC (or relatively low differentiation among RILs for GPC) compromised the significance of their association. A significant association was not found between mean GPC and HI (Table 2; Fig. 2).

QTL mapping

The combined AFLP and SSR map was used for composite interval mapping (CIM), and 142 QTLs were detected for six quality traits across environments (Supplemental Table 2). Among them, 71 QTLs (50%) were found in the A genome, 44 (31%) in the B genome, and 27 (19%) in the D genome. Most of the QTLs identified for KW, KD, TW, and GPC were associated with genomes A and B. The QTLs for HI were associated with genome D, as expected,

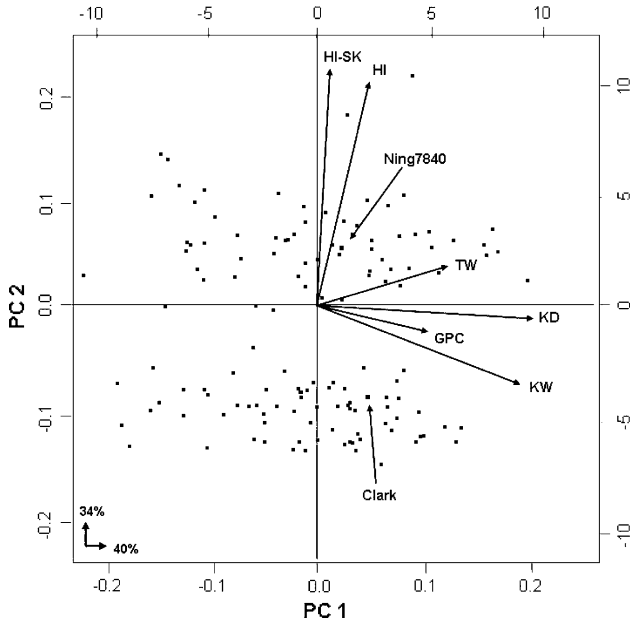

Fig. 2 Principal component analysis (PCA) biplot summarizing the relationships among wheat milling traits and class factors for the RIL population Ning7840 × Clark evaluated in various Oklahoma environments from 2001 to 2003. Traits are test weight (TW), kernel weight (KW), kernel diameter (KD), grain protein content (GPC), NIR hardness index (HI), and SKCS hardness index (HI-SK)

but also with genome B. With exception of HI, all quality traits in this study showed a weak association with the D genome. The respective number of QTLs from homoeologous groups one to seven was 12 (9%), 10 (7%), 10 (7%), 21 (15%), 48 (34%), 26 (18%), and 14 (10%), respectively. Given that a QTL in the same chromosome location was considered a consistent putative QTL for a trait if significant in at least two environments, the number of consistent QTLs ranged from six to eight for milling factors and two to four for class factors (Table 1).

Description of QTLs for quality traits

For TW, QTLs were located mainly on chromosomes 1DL, 2DL, 4AS, 4B, 5AS, 5AL, 5BS, and 6AS (Table 1). Phenotypic contributions of all QTLs ranged from 33 to 69% and varied with experiments. A QTL on the short arm of chromosome 5A showed a significant effect on TW with LOD values ranging from 3.1 to 5.6 in four environments that spanned all three locations in different years. The SSR marker interval *Xgwm154-Xgwm156* covered the QTL across environments. The QTLs on chromosomes 1DL, 4AS, and 5BS were consistently detected in three of the seven environments. Four additional QTLs on 2DL1, 4B, 5AL, and 6AS significantly affected TW in two environments. Among the eight QTLs, the Ning7840 alleles, except two QTL on 5AS and 6AS, increased TW.

Phenotypic variation for KW and KD was highly informative in this population, evidenced by the relatively long trait vectors in the biplot (Fig. 2). For KW, we identified major QTL regions on chromosomes 1BS, 4B, 5AS, 6AS, and 7AL (Table 1; Fig. 3). These QTLs together explained 47–73% of the phenotypic variance for KW in different experiments. The most consistent QTLs for KW (significant in all five environments where kernel weight was measured) were identified on chromosomes 6AS (LOD = 3.1–6.1) and 7AL (LOD = 5.7–11.6) and located in the intervals *Xwmc398-Xgwm132* and *Xgctg.gtg4-Xgwm332,* respectively. An additional putative QTL for KW was detected on 6AS and showed a significant effect in four of the five environments. The Clark alleles for these three major QTLs had a positive effect on KW. Four other QTLs (two on 1BS and one each on 4B and 5AS) were detected in two to three environments (Table 1) and may also constitute important QTLs for TW. Among these four QTLs, only the one on 5AS showed a positive effect from the Clark allele on KW.

For KD, six QTLs were identified on chromosomes 4AL, 5AL, 5AS, and 6AS, and together explained 42–71% of the phenotypic variation in different experiments (Table 1). Among these, common QTL regions were identified for KW and KD on chromosomes 5AS and 6AS (two QTLs each; Fig. 3), as would be expected given their strong phenotypic relationship.

Although there was no difference in mean GPC between Clark and Ning7840 (136 g kg^{-1}), the RILs varied significantly from 123 to 157 g kg^{-1} (Supplemental Table 1 and Fig. 1). With this level of transgressive segregation, two QTLs were detected on chromosomes 3AS and 4B (Table 1) and explained 19–36% of the phenotypic variance for GPC in different experiments. Alleles from Ning7840 contributed to increased protein content at both loci. The QTLs on 4B also affected KW and TW (Table 1). Another QTL on 3AS was unique to GPC from Ning7840. Positive QTLs for GPC from the SRW parent Clark were not detected.

The bimodal distributions observed for both measurements of HI (Fig. 1) indicate that this population of RILs contained two distinct hardness classes, based on either differential particle size (NIR) of uniformly ground whole wheat samples or resistance to crushing (SKCS). Two putative QTLs on the short arm of chromosome 5D and the long arm of chromosome 5B were associated with NIR hardness index (HI; Table 1). The 5DS QTL allele from hard wheat parent Ning7840 increased hardness and explained 70–77% of the phenotypic variation with LOD values from 31.7 to 61.7, whereas another QTL on 5DL showed only marginal significance for NIR hardness (R^2 = 3%). The same 5DS QTL also had a major effect (R^2 = 71.2–85.4%) on HI-SK. This QTL peaked at the

Table 1 Consensus genomic regions and their associated additive effects of significant QTLs for wheat quality factors identified by composite interval mapping across at least three environments

Linkage group	No. of locations	Position (cM)	Marker interval	LOD	a[a]	R^2 (%)
Test weight						
1DL	3	2–30	Xgwm191–Xcfd19	3.6–4	0.61–0.84 kg hL^{-1}	10.2–11.1
2DL	2	27	Xwmc168–Xgwm382	3.0–3.1	0.46–0.55 kg hL^{-1}	8–9.1
4AS	3	0–12	Xbarc206–Xbarc138	3–4.8	0.50–0.87 kg hL^{-1}	8.1–12.3
4B	2	79–82	Xgwm368–Xwmc617	2.7–5.7	0.55–0.80 kg hL^{-1}	8.3–16.4
5AS	4	25–48	Xgwm154–Xgwm156	3.1–5.6	−(0.50–0.75) kg hL^{-1}	8.1–15.5
5AL	2	40	Xwmc524–Xcfa2149	4–6.7	0.6–0.74 kg hL^{-1}	9.4–19.3
5BS	3	15–20	Xgwm68–Xbarc216	3–6.3	0.54–0.71 kg hL^{-1}	7.6–16.9
6AS	2	58–60	Xgwm132–Xwmc807	3.1–3.7	−(0.47–0.61) kg hL^{-1}	7.8–9.5
Total						32.6–68.6
h^2			0.62 (0.51–0.73)[c]			
Kernel weight						
1BS	2	13	Xgwm403–Xbarc61	3.4–4.1	0.77–0.81 mg	6.5–9.0
1BS	3	22	Xcfd20–Xgwm131	3.4–4.2	0.78–0.84 mg	6.8–9.4
4B	3	72–74	Xgwm368–Xwmc617	2.9–4.5	0.6.4–0.71 mg	6.2–12.3
5AS	3	26–37	Xgwm154–Xbarc180	2.6–4.3	−(0.66–0.84) mg	5.8–7.8
6AS	5	48–53	Xwmc398–Xgwm132	3.1–6.1	−(0.66–1.04) mg	5.8–14.6
6AS	4	61	Xbarc1055–Xwmc807	2.9–5.6	−(0.76–1.01) mg	5.7–14.2
7AL	5	4–9	Xgctg.gtg4–Xgwm332	5.7–11.6	−(1.16–1.57) mg	17–21.5
Total						47.0–73.0
h^2			0.73 (0.65–0.79)[c]			
Kernel diameter						
4AL	2	21	Xwmc313–Xwmc307	4.8–6.3	0.05–0.06 mm	11.6–12.4
5AL	4	41	Xwmc524–Xcfa2149	2.2–10.1	0.03–0.07 mm	4.6–18.5
5AS	3	15–19	Xctcg.ctg2–Xaca.cta4	2.6–6.8	−(0.03–0.06) mm	8.1–14
5AS	4	27–36	Xgwm154–Xgwm415	4.9–6.4	−(0.05–0.06) mm	8.6–13.7
6AS	4	43–47	Xctcg.gac1–Xwmc398	3.6–7.9	−(0.04–0.06) mm	6–16.2
6AS	4	52–55	Xwmc398–Xgwm132	2.8–5.9	−(0.04-0.06) mm	6.9–15.8
Total						42.2–71.3
h^2			0.71 (0.63–0.78)[c]			
Grain protein content						
3AS	2	21	Xwmc749–Xgwm369	4–4.7	0.27–0.28 g kg^{-1}	9.4–11.2
4B	3	74–87	Xgwm368–Xwmc617	2.8–5.2	0.15–0.29 g kg^{-1}	8.3–16.8
Total						19.1–35.6
h^2			0.50 (0.36–0.62)[c]			
NIR-hardness index					0–100[b]	
5DS	5	1	Pinb/Xcfd18	36–50.2	12.27–18.93	69.7–76.8
5DL	2	1	Xgwm212–Xcfd29	2.7–3	−(2.41–2.53)	2.8–3.3
Total						75.9–88.7
h^2			0.91 (0.88–0.93)[c]			
SKCS-hardness index					0–100[b]	
1DL	2	18	Xaag.ctc2–Xwmc429	5.3–6.6	−(5.28–5.59)	5.8–6.5
5BL	4	3–4	Xaca.cta13–Xwmc289	3–3.9	3.55–4.48	2.9–5.1
5DS	5	1	Pinb/Xcfd18	37.7–61.7	14.58–21.18	71.2–85.4
7AL	3	8–12	Xgctg.gtg4–Xgwm332	2.9–4.8	4.2–5.4	3.4–5.4
Total						83.3–89.5
h^2			0.96 (0.95–0.97)[c]			

[a] Additive effects were estimated as the mean (in trait unit) difference between the two RIL genotypic groups carrying the Clark and Ning7840 alleles. A positive value implies the Clark allele increased phenotypic value, whereas a negative value implies the Clark allele decreased phenotypic value

[b] 0 = Extremely soft, 100 = extremely hard

[c] Numbers in parenthesis are confidence interval of heritability

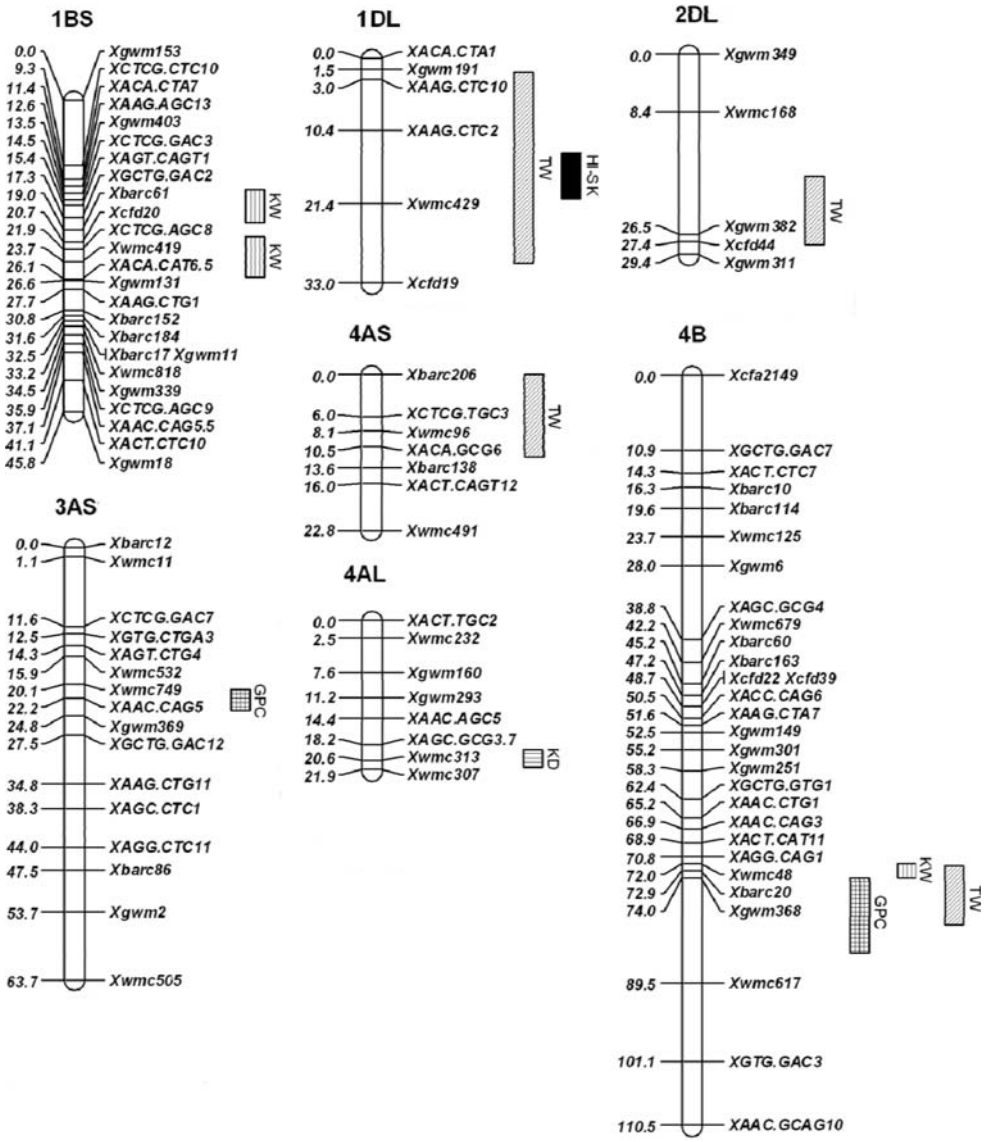

Fig. 3 Primary genomic regions of identified major QTLs (LOD > 3) affecting wheat milling traits and class factors for the Ning7840 × Clark RIL population evaluated in various Oklahoma environments from 2001 to 2003. *Bars* indicate marker interval of QTL for different traits

Pinb-D1 locus. Besides this QTL, three minor QTLs on 1DL, 5AS, and 7AL were identified for HI-SK, explaining 3–7% of the phenotypic variance (Table 1).

Discussion

A high-density linkage map for QTL mapping

Marker coverage of the genome is crucial for QTL detection. If markers closely linked to a QTL are not mapped, a QTL scan may not detect the QTL. Working against the requirement for adequate marker coverage is the immense size of wheat genomes and low genetic polymorphism among cultivars. Thus, it is difficult to construct a high-density map for QTL discovery in a single population derived from two wheat cultivars. In several previous quality trait mapping studies, maps used for QTL scanning contained 100–250 markers and did not cover all chromosomes (Groos et al. 2003; Huang et al. 2006; Zanetti et al. 2001). To improve genome coverage within our mapping population, we used two genetically diverse parents, Ning7840 from China and Clark from USA. We screened all publicly available SSR primers for polymorphism between the two parents and more than 100 AFLP primer combinations to fill gaps between SSR markers, and located about 1,000 markers in the initial map. Because overlapping or tightly linked markers may not provide additional information for the QTL scan and may increase calculation complexity, they were removed from the final map. The final map for QTL analysis consisted of 594 markers covering all 21 chromosomes at a total of 2,200 cM genetic distance. Therefore, this genetic map provided high genome coverage and a powerful tool for QTL analysis.

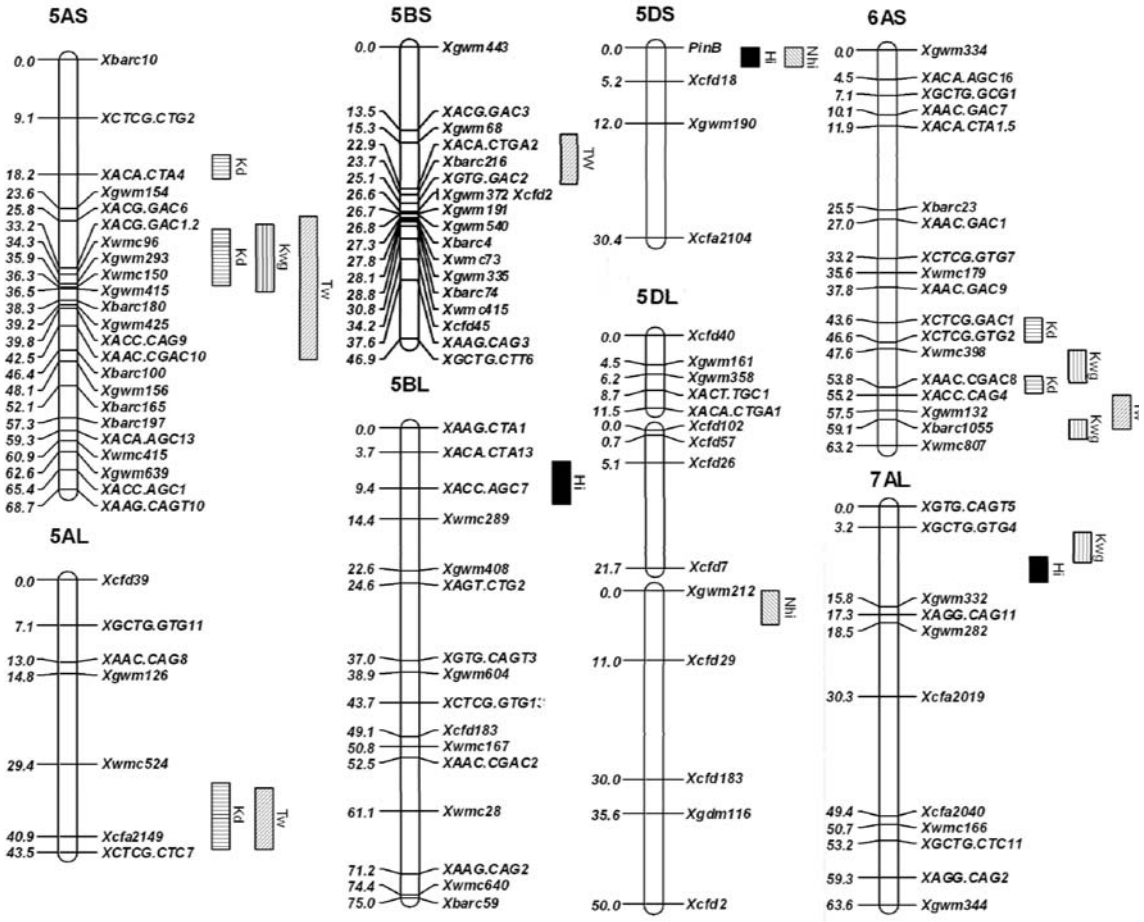

Fig. 3 continued

Using this new map, we identified a total of 15 chromosome regions that showed repeatable associations with at least one of the six quality traits over at least two testing environments. The number of QTLs detected for each trait varied from two (GPC and HI) to eight (TW), with most QTLs showing minor effects on the quality traits evaluated.

QTL for wheat class factors

One of the most widely studied quality traits in wheat is protein content, for which QTLs on chromosomes 2B and 2D were reported by Prasad (1999) and Campbell et al. (2001). The most widely reported QTLs were on chromosomes 5D, 5A, and 6B (Distelfeld et al. 2004; Khan et al. 2000; Olmos et al. 2003; Zanetti et al. 2001). In this study, two QTLs for GPC were identified on chromosomes 3AS and 4B. We could not determine whether this QTL is the same as the one on 3A previously reported by Groos et al. (2003), because common markers were not identified between the two maps. Another QTL on chromosome 4B was detected in three environments. This new QTL was not reported previously and may have potential for improving hard wheat GPC in breeding programs. The two QTLs for GPC identified in this study explained no more than 17% of the phenotypic variation. Thus, other still unidentified QTLs, if not environmental effects (which are known to be important; Groos et al. 2003) may be responsible for the remaining portion of the phenotypic variation. Clark had protein content similar to that of Ning7840, but no positive alleles for protein content were detected from the soft parent Clark. The gene for GPC on 6BS (Uauy et al. 2006) was not detected in the study.

Hard wheat genotypes tend to have higher GPC than soft wheat genotypes (Bushuk 1998; Wrigley et al. 2009), reflecting long-term divergent selection pressures between hardness classes. However, Ning7840 and Clark shared the same GPC with contrasting hardness. The major hardness QTL was mapped on 5DS in the absence of any neighboring GPC QTL in the D genome. Also, no association was found between GPC and kernel hardness across all RILs differing widely in HI (Fig. 2). When the RILs were grouped on the basis of relatively high (>40 HI-SK) and low (≤40 HI-SK) HI, mean GPC of the hard RILs across environments was only 2 g kg^{-1} greater (0.2% units,

$P > 0.05$) than that of the soft RILs. This may be due to the wide variation ($P < 0.05$) observed for wheat protein within each hardness group. Within groups, the harder RILs showed significant correlation for HI versus GPC, which is consistent with Carver (1994), whereas no significant correlation was detected within the softer group.

Although several QTLs were reported for kernel hardness (Sourdille et al. 1996), it is well known that allelic differences at a single locus may lead to hardness class differences (Giroux and Morris 1998). *Pina-D1* and *Pinb-D1* on the short arm of chromosome 5D largely represent the genetic basis of hardness variation (Giroux and Morris 1998; Morris 2002). Our study attributed a major effect for kernel hardness to the same gene in the Ning7840/Clark population. Further screening using *Pina-D1* and *Pinb-D1* gene markers confirmed that the *Pinb-D1* mutation (*Pinb-D1b*) accounted for harder kernels of Ning7840 and explained 70–85% of the phenotypic variation for kernel hardness. Besides the *Pinb-D1* locus, a QTL on 5DL influenced NIR HI, and QTLs on 1DL, 5BL, and 7AL showed minor effects on HI-SK. All were minor QTLs or modifiers for hardness with significant effects in two to four testing environments. The QTLs were different from those reported previously (Campbell et al. 1999; Sourdille et al. 1996). Therefore, *Pina-D1* and *Pinb-D1* are key loci for hardness, but other minor QTLs may modify the expression of these loci in various genetic backgrounds and environments.

QTL for wheat milling factors

Eight QTLs were identified from seven chromosomes in this study. Four of the QTLs (on 1DL, 4AS, 5AS, and 5BS) showed a significant effect on TW in at least three environments and thus were the more consistent QTLs for TW. To our knowledge, very few molecular mapping studies have addressed TW, a critically important trait that determines economic value from the farmer to the miller. The QTLs on 2D, 4A, and 5A were coincident with QTLs reported by Campbell et al. (1999) and Huang et al. (2006). The QTLs on 6B and 7A reported by Galande et al. (2001); Huang et al. (2006), and Elouafi and Nachit (2004) were not detected in this study.

Lately, several attempts have been made to understand the genetic basis of KW. Chromosome regions associated with KW on 5AL were reported by Campbell et al. (1999); on 2B, 3B, 4B, and 6B by Ammiraju et al. (2001), Elouafi and Nachit (2004), Groos et al. (2003), and Varshney et al. (2000); and on 2D, 4B, 4D, and 6A by Huang et al. (2006). In this study, seven QTLs on five chromosomes showed significant effects on KW in at least three environments, with one exception (1BS1 in two environments). Among these five QTLs, two (6AS and 7AL) showed consistent effects on KW in all five environments. The QTL on 7AL was most evident by accounting for 17–22% of the phenotypic variation among the five environments, and thus we consider it a major QTL for KW and a good candidate for marker-assisted selection. Of the seven putative QTLs for KW, Clark contributed positive effects except for the QTLs on 1B and 4B. The QTL on 6A was in the same chromosome region as previously identified (Huang et al. 2006) based on common markers in both maps. However, we could not confirm whether the QTL on 4B was the same as reported by Huang et al. (2006) or Elouafi and Nachit (2004), because common markers were not found between the two maps. QTLs on 5AS and 7A for KW were reported by Börner et al. (2002) and Cuthbert et al. (2008), respectively.

Six putative QTLs associated with KD were detected in linkage groups 4AL, 5AL, 5AS, and 6AS (Table 1; Fig. 3). The Clark allele increased KD for four of the six QTLs. The QTL regions on 5A and 6AS were the most consistent across environments. Chromosome 5A contained three QTLs with two closely linked QTLs on 5AS, and 6AS also contained two closely linked QTLs. The QTL on 4AL identified from two environments was uniquely associated with KD. Our findings coincided with earlier reported QTLs on 5A (Campbell et al. 1999), but neither of the QTLs reported by Dholakia et al. (2003) on 2BL and 2DL were identified here.

Genetic relations among quality traits

Yamazaki and Briggle (1969) and Marshall et al. (1984) described the components of TW as KW (density of the grain) and kernel morphology (packing efficiency of the grain). Differences in kernel morphology may modify the association of TW and KW. Previous studies in bread wheat on the correlation of these factors varied from positive (Gibson et al. 1998) to slightly negative (Schuler et al. 1994). The bi-trait correlations in Table 2 may be extended to view multi-trait relationships within the inference space of RIL variation by using the PC-biplot (Fig. 2). This biplot elucidates two important genotype × trait trends: the strong association of PC1 with kernel size factors (KD and KW) and the separation of two distinctive clusters of genotypes by PC2 according to HI. Kernel diameter and KW showed a strong positive association in the biplot, as did TW and KD. Protein content showed a close association with KW, but the relatively short vector for wheat protein (i.e., relatively low differentiation among RILs for wheat protein) compromised the interpretation of their association.

As may be expected from the high phenotypic correlations, several of the markers associated with TW on 5AS and 6AS were also significantly associated with KW and KD (Table 1). Moreover, the marker intervals

Table 2 Phenotypic correlation coefficients for wheat milling traits and class factors for the RIL population, Ning7840 × Clark, evaluated in various Oklahoma environments from 2001 to 2003

Traits	KW	HI-SK	KD	HI
HI-SK	−0.30*			
KD	0.88*			
HI		0.94*	0.20*	
GPC	0.32*		0.35*	
TW	0.43*	0.20*	0.50*	0.30*

Traits are test weight (TW), kernel weight (KW), kernel diameter (KD), grain protein content (GPC), NIR hardness index (HI), and SKCS hardness index (HI-SK). Only the significant r values ($P < 0.05$) are shown in the table

Xbarc18-/Xgwm154 and *Xwmc807-Xwmc398* were consistently identified as common chromosome regions for all three traits (Fig. 3), with Clark contributing positive effects. Therefore, markers from these regions are good candidates for marker-assisted selection, which may facilitate selection for larger and heavier kernels.

Contrary to the similarity in TW between the two parents (mean difference of 0.4 kg hL^{-1} across environments), kernel morphology differed noticeably. Kernels of Ning7840 were narrow and long, whereas kernels of Clark were short and rounded (plump). The QTL on 5AL may influence packing efficiency through its effect on kernel morphology because that was the only distinctive contribution of Ning7840 to both higher TW and larger KD, at least with respect to linkage group 5AL. To test this hypothesis, we classified the RILs on the basis of the marker *Xcfa2149* that was in most consistent marker interval on 5AL (*Xwmc524-Xcfa2149*) for the presence or absence of the Ning7840 allele. Consistent with kernel characteristics described by Briggle and Reitz (1963), kernels of RILs with the Ning7840 allele exhibited a crease with narrow width and shallow depth and a tendency toward angular checks and oval shape. Kernels of RILs without the Ning7840 allele, however, had mid-wide and mid-deep crease, rounded cheeks, and a tendency toward ovate shape. These patterns were consistent across all environments in which kernel samples were available (five of seven environments). To further support these visual observations, TW and KD were compared between marker groups. The RILs with the Ning7840 alleles exceeded those without by 1.06 kg hL^{-1} ($P < 0.001$) for TW and by 0.09 mm ($P < 0.001$) for KD.

In summary, our QTL analysis accounted for packing efficiency variation through the intervals (*Xbarc180-Xgwm154* on 5AS and *Xwmc524-Xcfa2149* on 5AL) relating to TW and KD in the both arms of chromosome 5A and chromosome 4B relating to KW and KD, and the short arm of chromosome 6A relating to all three packing efficiency traits. Important QTL co-localization was observed between KW and grain yield (Marza et al. 2006) in linkage groups 4B (*AGG.CAG1-AAC.GCAG4*) and 5A. This has important implications for simultaneous improvement of milling yield and grain yield (Marshall et al. 1984; Schuler et al. 1994). With the additional QTLs detected for wheat protein on chromosomes 4B and 3AS, the consistency of quality factor QTLs across environments reveals their potential for marker-assisted selection.

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