MAPPING QUANTITATIVE TRAIT LOCI FOR AGRONOMIC AND QUALITY FACTORS IN WHEAT

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CHAPTER I

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QTL FOR YIELD AND RELATED TRAITS IN THE WHEAT POPULATION, NING7840 x CLARK

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ABSTRACT

Grain yield and associated agronomic traits are important factors in wheat (*Triticum aestivum* L.) improvement. Knowledge regarding the number, genomic location, and effect of quantitative trait loci (QTL) would facilitate marker-assisted selection and the development of cultivars with desirable characteristics. Our objectives were to identify QTLs directly and indirectly affecting grain yield expression in the Southern Great Plains of the USA. A population of 132 F_{12} recombinant inbred lines (RILs) was derived by single-seed descent from a cross between the Chinese facultative wheat Ning7840 and the US soft red winter wheat Clark. Phenotypic data were collected for 15 yield and other agronomic traits in the RILs and parental lines from three locations in Oklahoma from 2001 to 2003. Twenty-nine linkage groups, consisting of 363 AFLP and 47 SSR markers, were identified. Using composite interval mapping (CIM) analysis, 10, 16, 30, and 14 QTLs were detected for yield, yield components, plant adaptation (shattering and lodging resistance, heading date, and plant height), and spike morphology traits, respectively. The QTL effects ranged from 7 to 23%. Marker alleles from Clark were associated with a positive effect for the majority of QTLs for yield and yield components, but gene dispersion was the rule rather than the exception for this RIL population. Often, QTLs were detected in proximal positions for different traits. Consistent, co-localized QTLs were identified in linkage groups 1AL, 1B, 4B, 5A, 6A, and 7A, and less consistent but unique QTLs were identified on 2BL, 2BS, 2DL, and 6B. Results of this study provide a benchmark for future efforts on QTL identification for yield traits.

Keywords: Wheat · QTL · Yield · Plant adaptation · Spike morphology · SSR · AFLP

INTRODUCTION

As the world's most important food crop, wheat (*Triticum aestivum* L.) is grown on over 208 million hectares, yielding 2665 kg ha^{-1}, and now producing over 556 million metric tons annually (FAO, 2004). Grain yield in wheat is determined concurrently by a number of plant and grain characteristics. These are complex quantitative traits controlled by several genes, expressed in progeny populations in continuous distributions, and highly influenced by environmental conditions (Kearsey and Pooni 1996). These factors make it difficult to define yield according to gene effect or gene number using classical quantitative genetic methods. The application of new molecular marker technologies for quantitative trait locus (QTL) analysis, such as amplified fragment length polymorphism (AFLP), simple sequence repeat (SSR), and single nucleotide polymorphism (SNP) markers, has provided an effective approach to dissect complicated quantitative traits into component loci to study their relative effects on a specific trait (Langridge et al. 2001; Doerge 2002).

Using single chromosome recombinant substitution lines and restriction fragment length polymorphism (RFLP) markers, QTLs for yield and important agronomic traits were identified on chromosomes 3A (Shah et al. 1999; Campbell et al. 2003), 4A (Araki et al. 1999), and 5A (Kato et al. 2000). Using a more saturated RFLP map derived from the population, Opata 85/W7984, (Borner et al. 2002) detected 64 QTLs for about 20 agronomic characters. Additional QTLs controlling other plant adaptation and morphology traits were reported, including heading date (Shah et al. 1999; Bullrich et al. 2002; Shindo et al. 2003), plant height (Cadalen et al. 1998; Huang et al. 2003, 2004), lodging (Keller et al. 1999), leaf rust reaction (Singh et al. 2000), and spike morphology (Sourdille et al. 2000; Borner et al. 2002).

The development of molecular markers for important wheat traits and their application in breeding programs is challenged by multiple genome constitution (AABBDD, allohexaploid and amphidiploid: 2n = 6x = 42) and a relatively large genome size of 16,000 Mbp, of which more than 80% is repetitive DNA (Roder et al. 1998; Marshall et al. 2001). One advantageous marker class for QTL detection in wheat might be AFLP markers, which amplify a large number of DNA fragments in a single PCR reaction, show a high level of polymorphism, and offer high reproducibility and reliability under stringent PCR conditions (Vos et al. 1995). Another important marker class is simple sequence repeat (SSR), also called microsatellites, which are stable, abundantly dispersed throughout the genome, and locus-specific in hexaploid wheat. Detailed SSR genetic maps are now available for wheat (Roder et al. 1998, 2002; Pestsova et al. 2000; Somers et al. 2004). Though SSR markers now are recognized for their efficiency in detecting a single locus with polymorphism of known identity, QTL mapping based exclusively on SSRs currently may be an unrealistic goal due to limited availability of SSR primers (Langridge et al. 2001). The creation of a 'skeletal' genetic map with SSRs, however, is achievable and serves a critical role in providing physical anchor points for specific chromosomes in a saturated AFLP map.

Identification of QTLs influencing grain yield and related traits is needed to more precisely define their inheritance. The vast majority of genomic-based research in wheat has previously focused on more simply inherited traits with indirect effects on productivity. The objectives of this study were to 1) dissect QTLs affecting grain yield in winter wheat based on AFLP and SSR markers, 2) determine the chromosome locations and phenotypic effects of these yield related QTLs, 3) identify molecular markers associated with these traits.

MATERIALS AND METHODS

Plant materials

A population of 132 F_{12} recombinant inbred lines (RIL) was derived by single-seed descent from the F_2 of the cross, Ning7840/Clark. Ning7840 is a Chinese hard red facultative cultivar with the pedigree, Avrora/Anhui 11//Sumai 3. It has relatively low yield potential but is highly resistant to various rust pathogens and *Fusarium graminearum* (Bai et al. 1999). Clark is a soft red winter wheat cultivar developed at Purdue University, IN, USA (Ohm et al. 1988). Distinctive features of Clark are its early date of heading combined with good yield potential, high kernel weight, and resistance to *Wheat soilborne mosaic virus* (Ohm et al. 1988).

Experimental design

Ning7840, Clark, and the 132 RILs were evaluated at one to three Oklahoma locations (Stillwater, 36°9'N and 97°05'W, Lahoma, 36°22' and 98°00', and Altus, 34°39' and 99°20') for each of three crop years ending in 2001, 2002, and 2003, using a replicates-in-sets design with three replications. Plot size was 1.4 m^2, and seeding rate was 58 kg ha^{-1}. All experiments were planted according to a grain-only management system (early Oct. to early Nov.), and fertilizer was added according to soil-test recommendations for a 4000 kg ha^{-1} yield goal.

Traits

In addition to grain yield, information on adult-plant characters was collected based on relevance to this mapping population and on level of trait expression (Table 1). Grain yield (GY) was measured as the weight of wheat grain harvested from the entire plot area. Spike number (SN) was calculated from the number of spikes present in two 50-cm row segments 23 cm apart. Kernel number $spike^{-1}$ (KS) and kernel weight $spike^{-1}$,

hereafter called spike weight (SW), were determined from the mean of 15 random spikes. Heading date (HD) was recorded as the number of days after 31 March when spikes were fully emerged from 50% of the plants in a plot. Physiological maturity date (MD) was recorded on a visual scale from 1 = early to 4 = late based on the appearance of a yellow peduncle at the base of the spike. Plant height (HT) was measured at harvest maturity from ground level to the tip of the spike, excluding awns. Shattering (SH) and lodging (L) were recorded at harvest maturity on a visual scale from 1 = no shattering or no lodging to 5 = severe shattering or lodging. Plant yellowing, indicative of barley yellow dwarf symptoms, was recorded from 10 to 30 April (heads emerged and during anthesis) using the scale from 1 = completely green canopy (no symptoms) to 5 = yellow canopy (severe symptoms). Leaf rust reaction (LR) was based on percent severity. Spike length (SL) was measured from base to tip, excluding awns. Spike density (SD) was rated on a scale from 1 = compact spike to 4 = lax spike. Chaff color (C) was recorded as dark (score of 1), intermediate (2), or light (3). Some trait measurements were restricted to two or three environments depending on their level of repeatability or expression (Table 1). Twenty-seven RILs which showed unusually high shattering were removed from the data analysis in 2003.

Analysis of SSRs

Total genomic DNA was isolated from young leaf tissue of 2 to 4 week-old greenhouse-grown plants of both parents (Ning7840 and Clark) and the 132 F_{12} RILs using the modified cetylmethylammonium bromide (CTAB) procedure (Saghai-Maroof et al. 1984). The PCR was performed in a volume of 12 μL containing 0.200 mM of each dNTP, 1x PCR buffer, three pmol of each primer, 2.5 mM $MgCl_2$, 1 U of *Taq* polymerase, and 50 ng DNA. The PCR was performed by means of a touchdown

program consisting of five cycles of 45 s at 95°C, 5 min of annealing at 68°C which decreased by 2°C each cycle, and 1 min at 72°C. In the following five cycles the annealing temperature started at 58°C for 2 min and lowered by 2°C per cycle. The PCR continued for 25 additional cycles of 45 s at 95°C, 2 min at 50°C, and 1 min at 72°C with a final elongation step of 72°C for 5 min. The PCR products were denatured for 5 min at 94°C before they were separated in a 6.5% polyacrylamide gel on a Li-Cor IR-4200 DNA sequencer (Li-Cor Inc., Lincoln, NE) using a fluorescent-labeled M13 primer for PCR detection. The SSRs screened in this study included 181 XGWMs (Roder et al. 1998), 160 BARCs (Cregan et al. 1999), 36 GDMs (Pestsova et al. 2000), 20 WMCs (Gupta et al. 2002), and 3 DUPWs (Du Pont, USA).

Linkage mapping

The two parents and the 132 RILs were previously characterized using AFLP markers (Bai et al. 1999), producing 618 polymorphic band readings (G. Bai, unpublished data). 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 missing (-) and later combined for constructing a genetic linkage map. Linkage analysis was performed using the MAPMAKER program (Macintosh V2.0, Lander et al. 1987). Recombination frequencies were converted to centimorgans (cM) using the Kosambi mapping function (Kosambi 1944).

Statistical analysis

The complete set of data from each environment was subjected to analysis of variance (ANOVA) to determine the main effects of genotype (RIL) and replication factors. Phenotypic correlations were calculated for all combinations of traits based on RIL means across environments. Principal component (PC) analysis of genotypes across

environments was performed based on standardized ($\mu = 0$, $\sigma = 1$) means data using the PRINCOMP procedure of SAS (SAS Institute 2003). Briefly, the resulting PC scores were represented in a genotype x trait biplot, trait vectors were drawn from the origin to the coordinates for each trait, and genotypes were represented by markers determined by their coordinates. An angle formed between two traits (or genotypes) approximated their correlation, with 0- and 180-degree angles indicating strong correlations and a 90-degree angle representing a weak or zero correlation (Yan and Kang 2003).

QTL analysis

The original set of marker data, the genetic map generated with MAPMAKER 2.0, and the phenotypic data were used in the QTL analysis. The Windows version of QTL Cartographer V2.0 (Wang et al. 2004) was used to conduct composite-interval mapping (CIM) analysis based on model 6 of the Zmapqtl procedure (Basten et al. 2001). The closest marker to each local LOD peak (putative QTL) was used as a cofactor to control the genetic background while testing at a position of the genome. The walking speed chosen for all QTL analysis was 2.0 cM. The LOD significance was estimated from 1000 permutations of the data. Additive effects of 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) using single-factor analysis from a general linear model procedure (Basten et al. 2001). For each QTL, R^2 was determined for the single marker closest to the identified QTL.

RESULTS AND DISCUSSION

Linkage map

A total of 400 SSR markers were screened, of which 82 (21%) were polymorphic between the parents. Combined with the 619 AFLP markers previously identified as polymorphic, 701 markers were subjected to linkage analysis. Twenty-nine linkage groups were constructed from 363 AFLP and 47 SSR markers, after removal of markers < 1 cM apart. Each group contained at least one anchor SSR marker (Fig. 1). This linkage map spanned 2,223 cM, with an average interval length of 5.4 cM. The recommended map distance for genome-wide QTL scanning is 10 recombinations per 100 meiotic events, or an interval length less than 10 cM (Doerge 2002). Of the 410 loci mapped, segregation distortion was detected for 28 AFLP and 2 SSR marker loci randomly distributed in different chromosomes.

Phenotypic summary

The phenotypic data were classified into three categories: yield traits, plant adaptation traits, and spike morphology traits (Table 1). The analysis of variance (data not shown) indicated a high level ($P < 0.01$) of genetic variation for all traits in all environments. Transgressive segregation was common among all traits (Table 1). Continuous distributions were also common except for shattering score. Test statistics for skewness and kurtosis were generally less than 1.0, indicating suitability of the data for QTL analysis.

Clark performed more favorably for yield and spike morphology traits, and Ning7840 showed greater resistance to leaf rust (Table 1). Mean grain yield, spike number, kernel number spike^{-1}, and spike weight were 9 to 26% greater for Clark than for Ning7840 across environments ($P < 0.05$). Clark also produced longer spikes than

Ning7840 in all environments ($P < 0.05$). Only for yield in Stillwater 2003 and for spike number in Stillwater 2001 did Ning7840 exceed Clark. Though genetic variation was found in the RIL population for all plant adaptation traits, Ning7840 and Clark did not differ for these traits, except for Clark's greater susceptibility to leaf rust. Parental differences were present, but inconsistent among environments, for heading date, shattering score, and lodging score.

Positive phenotypic correlation coefficients were found between each of the three yield components and grain yield (Fig. 2). As expected, greater shattering, lodging, plant yellowing, and leaf rust susceptibility were associated with lower yield. Hence, identification of QTLs with direct effects on yield requires scanning for QTLs that influence yield independently of these adaptation traits. Differences in the degree of spike compactness did not correlate with differences in grain yield, although more compact spikes made shorter spikes. From the biplot (Fig. 3), vectors representing uncorrelated traits formed 90-degree angles (e.g., GY vs. SD or HT), whereas highly correlated traits formed either acute (positive association; e.g., LR, Y, and L) or obtuse (negative association; e.g., GY vs. LR, Y, L, or SH) angles. In general, the biplot produced four distinct trait clusters indicative of strong positive association within clusters: GY and SW; KS and SL; LR, Y, and L; and HT, SN, and HD. Spike weight showed the strongest positive association with grain yield, which might be expected considering that spike weight integrates the effects of kernel number spike^{-1} and kernel weight. Furthermore, given the breadth of environments for which yield and spike weight were associated, mapping of these traits could reveal consistent QTLs across variable environments.

QTL mapping

The composite-interval mapping analysis produced a total of 206 putative major and minor QTLs (Table 2, Fig. 1). For all categories of traits, QTL frequency was highest in the B genome with 124 QTLs (60%); another 64 (31%) and 18 (9%) QTLs were found in genomes A and D, respectively. Distribution of QTLs was balanced among homologous chromosome groups one to seven as follows: 25 (12%), 33 (16%), 34 (17%), 25 (12%), 29 (14%), 36 (17%), and 24 (12%). Chromosomes 2A, 3D, and 4D were not included in the analysis.

We detected a mean of six putative QTL for yield related traits, four for plant adaptation traits, and five for spike morphology traits. These results coincide with a summary of 47 studies on cereals, where the number of QTLs identified for a particular trait varied up to about 16 with a mean of about 4 (Kearsey and Farquhar 1998).

QTLs for yield traits

Ten QTLs were detected with a major effect on grain yield (Tables 2 and 3) and with a high degree of gene dispersion between the parents. The Clark allele increased grain yield for five QTLs in linkage groups 2BL, 4AL, 4B, 5A, and 6B, with LOD values of 3.2 to 6.0 and accounting for 8 to 19% of the phenotypic variation. Alleles from Ning7840 increased yield at the other five major QTLs in linkage groups 1AL, 1B, 5B, 7A, and 7DL2, with LOD values from 3.1 to 7.0, accounting for 9 to 21% of the phenotypic variance.

Chromosome 5A, where our most repeatable yield QTL was identified, is known to carry a number of influential genes affecting anthesis date, frost tolerance, drought tolerance (Shindo et al. 2002; Toth et al. 2003), productivity, and adaptability (Kato et al. 2000; Huang et al. 2004). The QTL in 5A identified here may be related to the one

detected for yield by Kato et al. (2000). The yield QTL in linkage group 4B was uniquely detected in this population, though this genomic region was coincidental to other adaptation traits (plant height and shattering) and to spike length (Fig.1). We found no previous report of a yield QTL on 4B.

Less consistent or environment-specific chromosome regions associated with yield were identified in linkage groups 2BL, 4AL, 5B, 6B, and 7DL2 (Fig. 1). Similar findings with yield were reported for 2BL and 5B (Huang et al. 2003), 4AL (Araki et al. 1999), and 6B (Huang et al. 2004). No QTL was previously reported on 7DL.

The lack of association between yield and spike number at ST01 and ST02 resulted in no common QTLs between them (Fig. 3). Inconsistent parental differences in spike number (Table 1) further hindered an attempt to detect meaningful QTLs for this yield component. Linkage group 3BS contained a major QTL for spike number that explained 12% of the phenotypic variance (Table 3). This finding agrees with the results of Huang et al. (2003), but Huang et al. (2004) reported another QTL for spike number on chromosome 1B that may correspond to the consistent minor QTL we detected in linkage group 1B (Table 2).

In contrast to spike number, eight major QTLs were detected for kernel number spike^{-1} (Table 3). Six of these were mapped to linkage groups 1AL, 1B, 2BS, 3BS, 4B, and 7BS2 at which the Clark allele increased kernel number spike^{-1}. Two other QTLs, with positive effects from Ning7840, were found in linkage groups 2DL2 and 6A. The major QTL in linkage group 6A was significant in all environments and coincident with the 6A minor QTL for yield (Fig. 1). In another unrelated population, Huang et al. (2004) identified a QTL in the same genomic position and with similar effects. Other important QTLs for kernel number spike^{-1}, *CTCG.CGAC6/CTCG.CT2* on 1AL and

ACT.CAT11/AGG.CAG1 on 4B, showed common effects with grain yield in some, but not all, environments (Table 3 and Fig. 1).

Distinct differences between parental lines for spike weight allowed the identification of seven major QTLs in as many linkage groups (Table 3). Four QTLs in linkage groups 2DL, 3BS, 5A, and 6B explained 10 to 13% of the phenotypic variation, in which the Clark allele increased spike weight. Three QTLs in which Ning7840 increased spike weight were located in linkage groups 1B, 2BL, and 3BL2, explaining 8 to 11% of the phenotypic variance. Putative QTLs in linkage groups 1B and 6B were among the most consistent across environments, yet we found no QTLs previously reported in those positions. Additional evidence of QTLs was reported by Huang et al. (2004) in chromosomes 3BS and 6A; by Araki et al. (1999) and Borner et al. (2002) in chromosome 4A, and by Kato et al. (2000) in chromosome 5A. The strongest phenotypic association exhibited by spike weight and yield (Fig. 3) may be reflected in the common marker interval in linkage groups 1B (*GCTG.GTG2/AAC.GAC10*) and 5A (*BARC180/ACG.GAC1.2*). No common locus was identified among other QTLs that mapped to the same chromosome (2BL and 6B). The role of these unique QTLs for spike weight to yield formation is not easily elucidated considering yield fluctuations are tempered by spikes with fewer heavy kernels or with more numerous lighter kernels.

Summarizing to this point, yield traits in this population were largely influenced by QTLs distributed among linkage groups 1AL, 1B, 2BL, 3BS, 4B, 5A, 6A, and 6B. Considering all traits (Table 3), a QTL for spike number, kernel number spike^{-1}, and spike weight mapped to the same position in the marker interval *XGWM533/CTCG.AGC1* (linkage group 3BS) as did a QTL for kernel number spike^{-1} and kernel weight in the marker interval *AGG.CTC13/CTCG.AGC9* (linkage group 1B)

and *AGT.CTG13/XGWM389* (linkage group 3BS). Concordance in genomic positioning signals a molecular basis for the phenotypic relationships summarized in Figure 3.

QTLs for plant adaptation traits

Genomic regions significantly associated with yield were also associated with traits conditioning adaptation. Clusters of yield-coincident QTLs were found in linkage groups 1B (lodging and leaf rust reaction), 4B (plant height), 5A (shattering, lodging, and leaf yellowing), and 7A (shattering). Coincidence of QTLs may indicate either single genes with pleiotropic effects or that the genomic regions associated with these QTLs harbor a cluster of linked genes associated with yield potential and adaptation.

Shattering and lodging scores, leaf yellowing, and leaf rust reaction associated negatively with grain yield (Fig. 2, 3). Expression of shattering was relatively light in three environments (ST02, ST03, and LA03), but distinctly more severe in LA02 and AL03. Across those five environments, six putative QTLs were found in linkage groups 4B, 5A, 6A, 6B, 7A, and 7DL (Tables 2, 3). Detection of these QTLs was highly inconsistent among environments, and most had moderate effect with LOD values ranging from 3.2 to 3.5. One notable exception was the QTL in linkage group 7DL identified in ST03, which exhibited a LOD value of 9.8 and accounted for 56% of the phenotypic variance (Table 3). Interestingly, this major QTL was easiest to detect in an environment that produced the lowest RIL population mean for shattering. Grain yield in this environment did not map to the same linkage group as did the shattering QTL. Grain yield, however, did map to the same position for regions in linkage groups 5A (*ACG.GAC1.2/ACG.GAC6*) and 4B (*AAC.CAG2/ACT.CAT11*; closest marker interval), but still only in isolated environments (ST02 and LA02, Tables 2 and 3). The only linkage group to which shattering was mapped in multiple environments was 6B, a

linkage group relatively unimportant to grain yield expression in this population. We have found no published precedent for a shattering QTL in wheat.

Three major QTLs for lodging score were identified in linkage groups 1B, 4AL, and 5A. The QTL in 5A was identified in two of three environments and a QTL in a similar location was reported by Keller et al. (1999). Chromosome 5A is also mentioned as one of the locations of a stem solidness gene (Cook et al. 2004). Among all traits plotted in Figure 3, lodging score showed the strongest negative association with yield. This relationship may in part be attributed to the consistent QTL on linkage group 5A, which mapped to the same chromosome region for both traits. For this region, the allele from Clark increased yield but decreased lodging score.

The leaf yellowing we observed immediately prior to heading was indicative of barley yellow dwarf symptoms, though this was not confirmed serologically. Six QTLs were detected across linkage groups 2BL, 2DL2, 3BS, 5A, 6A, and 7DL3. Marker-assisted selection for resistance to *Barley Yellow Dwarf Virus* (BYDV) was previously attempted (Henry et al. 2000) based on microsatellite marker *XGWM37* located also on 7DL. We identified a single QTL on 7DL3 (LA03). The QTLs for leaf yellowing and yield coincided in a genomic region in linkage group 5A. Marker alleles associated with this locus had inverse effects on yield versus leaf yellowing.

Three major QTLs on 3BS, 1B, and 2DL2 were associated with leaf rust reaction. The QTL on 3BS (*XGWM493/ACT.TGC7*) was previously associated with *Lr*34*/Yr*18 (Singh et al. 2000).

Spike development and date of heading in wheat are considered to be controlled by three major groups of genes: photoperiod response genes on 5A and 5D; vernalization response genes on 5A, 5B, and 5D; and ‘earliness per se’ genes on homoeologous groups

2 and 4, 3A, 6B, and 7B. (Shah et al. 1999; Bullrich et al. 2002; Shindo et al. 2003). All QTLs identified in this population for heading date, except the linkage group in 3BL2, could be traced to those same chromosomes. The QTL on 5B, detected in three of the five environments (Table 2), was most consistent though two major QTLs could be detected from other linkage groups (3BL2 and 6B) in certain environments. Hence, heading date differences were likely driven by a combination of developmental factors in this population. The Ning7840 allele always delayed heading date for all QTL. Some of the same linkage groups harboring QTLs for heading date also influenced maturity date (5B and 6B). One major QTL unique to maturity date was detected in linkage group 1B, indicating independent mechanisms controlling maturity.

Six putative QTLs influenced plant height, but QTLs on 4B and 6A were the most consistent as they were detected in most environments. These regions have been widely reported elsewhere (Cadalen et al. 1998; Borner et al. 2002; Huang et al. 2003, 2004). The Clark allele on 6A increased plant height, but the Clark allele on 4B reduced it, owing to the complexity of genetic control of plant height. We found no significant association between yield and height in this population to warrant the consideration of height QTLs to indirectly manipulate yield (Fig. 1 and 3). However, a common marker interval was identified in linkage group 4B (*ACT.CAT11//AAC.GCAG4*; Table 3), in which the allele from Clark increased yield but decreased plant height.

Spike morphology

Nine major QTLs were identified for spike length. Those in linkage groups 1AS, 2BL, 2BS, 4B, and 7A showed a positive effect from the Clark allele, whereas QTLs on 1AL, 1B, 3BL, 5B, and 7BS showed a negative effect. The QTL on chromosome 3BL was detected in every environment (Table 2), although this chromosome rarely

contributed to grain yield variation. Only the QTLs identified on 1AS and 2BS were consistent with previous results (Sourdille et al. 2000). Contrary to their moderate phenotypic correlation coefficient, the degree of spike compactness, or spike density, was mostly dissociated with spike length based on coincidence of QTLs. Four QTLs affecting spike density were identified in linkage groups 1B, 4AL, 7BS, and 7DL3. Only the QTL on 7BS was found in the same position (*AGC.GCG13/AGG.CT3*) for both traits (Fig. 1).

Despite distinct differences in awn presence between parents (Clark, awnletted; Ning7840, fully awned), we were not able to reproduce findings of earlier studies for major QTLs on 4A and 6B (Sourdille et al. 2002); instead, we did identify one major QTL in linkage group 7BS2. Chaff color was attributed to genes on homologous group-1 chromosomes in an earlier study (Borner et al. 2002). We identified a major QTL for chaff color in linkage group 1B with darker color contributed by Clark. With a LOD value of 40 this QTL explained 45% of the total variability. The flanking interval for this QTL was *ACT.CAGT1/ACA.CTA8*.

Summarizing across all traits, the identified QTLs in each linkage group influenced, on the average, three traits. The QTLs for an unusually high number of traits were located on the linkage group 1B (eight from fifteen possible). Ning7840 is believed to possess the 1RS.1BL translocation (NGRP 2004), which was likely segregating in this RIL population. The 1RS.1BL translocation from Avrora was previously shown to increase grain yield in Oklahoma by 9 to 10% (Carver and Rayburn 1994), but only in one environment (ST03) was a QTL directly attributed to yield in linkage group 1B (Table 2).

In conclusion, the genetic control of grain yield and associated agronomic traits of wheat was dissected into QTLs. These traits were primarily influenced by QTLs concentrated in at least seven distinct genomic regions. Key QTLs in linkage groups 2BL, 2BS, 2DL, and 6B were uniquely associated with yield and yield components and offer the greatest potential for marker-assisted yield improvement schemes. In addition to 1B, other major QTLs in linkage groups 1AL, 4B, 5A, 6A, and 7A impacted grain yield through their effect on related traits (e.g., lodging resistance). Several important interval markers were AFLPs and will thus need to be converted into sequence-tagged site (STS) or more SSR markers need to be identified in these regions. With further validation, the identified QTLs for yield and agronomic related traits should allow the design of appropriate marker-assisted selection strategies that center on multi-trait selection for desirable characters with coincident QTL locations and on breaking unfavorable linkages between negatively correlated traits.

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Table 1. Phenotypic summary of yield related traits, plant adaptation traits, and spike morphology for Ning7840, Clark, and their RIL progeny evaluated in various Oklahoma environments from 2001 to 2003 (environments listed for each trait in decreasing order for RIL mean yield)

Trait	Env.	Parents		RIL Population†					
		Clark	Ning7840	Mean	Max.	Min.	SD	Skew-ness	Kurto-sis
Yield									
Grain yield (kg ha^{-1})	LA03	5089	4360	4074	5741	2089	803	0.03	-0.29
	ST03	3579	3777	3491	5686	2345	535	0.68	1.91
	ST01	2725	2381	2308	3616	431	641	-0.50	0.16
	AL02	2038	1892	1865	3247	381	555	-0.04	-0.02
	ST02	1947	1585	1691	3001	412	483	-0.10	0.10
	LA02	1481	953	1628	3880	186	826	0.47	-0.36
	AL03	1304	589	1241	3236	260	739	0.51	-0.79
Spike number	ST01	456	494	442	690	270	71	0.36	0.48
	ST02	721	539	608	955	387	98	0.34	0.42
Kernel number spike^{-1}	LA03	33.7	32.6	31.4	44.7	24.3	3.8	0.73	0.80
	ST03	38.0	34.6	37.2	51.3	28.7	4.3	0.64	0.62
	ST01	37.0	33.5	36.8	52.0	23.0	5.2	0.09	0.57
	AL02	33.7	30.3	29.1	43.7	10.0	6.5	-0.30	-0.29
	ST02	36.7	31.3	32.5	41.7	22.0	4.0	0.12	-0.55
Spike weight (g)	LA03	1.11	0.91	0.97	1.27	0.70	0.12	0.25	-0.28
	ST03	1.23	0.96	1.10	1.40	0.83	0.12	0.06	-0.46
	ST01	1.20	0.80	1.12	1.50	0.70	0.15	-0.45	0.10
	AL02	0.90	0.70	0.72	1.13	0.23	0.17	-0.25	0.18
Plant adaptation									
Heading date‡	LA03	24	22	25	34	19	4.6	0.31	-1.25
	ST03	27	27	28	34	22	2.5	-0.27	0.32
	ST02	21	25	23	27	16	3.0	-0.62	-0.44
	LA02	20	29	25	32	18	3.4	-0.09	-0.91
Maturity date (1-4) ¶	ST03	1.7	1.7	2.2	4.0	1.0	0.8	0.19	-1.08
	ST01	1.5	1.5	1.6	4.0	1.0	0.7	1.02	0.19
Plant height (cm)	LA03	86	85	85	102	67	8	-0.28	-0.46
	ST03	78	78	78	93	59	7	-0.16	-0.21
	ST01	71	70	67	88	48	7	0.15	0.23
	ST02	82	75	79	98	62	7	-0.02	-0.19
	LA02	83	72	79	103	55	9	-0.15	-0.04

Trait	Env.	Parents		RIL Population†					
		Clark	Ning7840	Mean	Max.	Min.	SD	Skew-ness	Kurto-sis
Shattering score (1-5)§	LA03	1.1	1.7	1.7	4.3	1.0	0.8	1.04	0.65
	ST03	1.2	1.1	1.2	3.7	1.0	0.4	2.97	11.56
	ST02	1.7	1.0	1.4	4.0	1.0	0.6	2.15	5.24
	LA02	3.5	2.0	2.2	4.0	1.0	1.2	0.41	-1.35
	AL03	3.0	4.3	3.4	5.0	1.0	1.6	-0.41	-1.44
Lodging score (1-5)††	LA03	1.0	1.2	1.7	5.0	1.0	0.9	1.45	1.77
	ST03	1.2	1.1	1.6	4.0	1.0	0.7	1.18	0.60
	ST02	1.3	1.7	2.4	4.0	1.0	0.8	0.00	-0.99
Leaf yellowing (1-5)‡‡	LA03	1.2	2.3	1.9	4.0	1.0	0.7	0.95	0.56
	ST03	1.9	1.8	1.9	3.3	1.0	0.5	0.63	0.36
	ST02	1.7	2.0	2.1	3.7	1.0	0.5	0.38	0.22
	LA02	1.3	2.3	2.1	4.7	1.0	0.8	1.09	1.77
Leaf rust reaction (%)¶¶	LA03	2.4	1.0	4.3	30.0	1.0	5.4	2.44	6.88
	ST02	46.0	12.7	45.9	93.3	1.3	27.5	-0.08	-1.30
	LA02	24.3	12.3	53.5	99.0	2.3	32.5	-0.13	-1.55
Spike morphology									
Spike length (cm)	LA03	8.1	7.7	8.0	10.0	6.0	0.75	0.16	0.17
	ST03	8.9	8.0	8.1	10.3	6.0	0.91	-0.17	0.16
	ST01	7.5	7.5	7.4	9.5	5.0	0.97	-0.11	-0.13
	AL02	8.0	6.7	7.7	9.7	5.7	0.86	0.24	-0.13
	ST02	8.0	6.3	8.2	10.7	6.3	0.91	0.57	0.09
Spike density (1-4)§§	LA03	3.0	2.0	2.8	4.0	1.0	0.6	-1.18	1.47
	ST03	3.0	2.3	2.8	4.0	1.0	0.7	-0.95	0.94
	ST01	4.0	3.0	3.4	4.0	1.0	0.8	-1.37	0.88

† Population of 132 F_{12} recombinant inbred lines
‡ Days after 31 March
¶ Early=1, late=4
§ No shattering=1, severe shattering=5
†† No lodging=1, severe lodging=5
‡‡ No yellowing=1, severe yellowing=5
¶¶ % severity
§§ Compact=1, lax=4

Table 2. QTLs detected in more than one environment (italicized) by composite interval mapping analysis for the Ning7840 x Clark RIL population evaluated in Oklahoma from 2001 to 2003 (bold = major QTLs, LOD > 3; non-bold = minor QTLs, 2 < LOD ≤ 3). QTLs detected only in a single environment are given in plain type. Environments arranged from left to right in decreasing order for RIL mean yield.

		Environments								
Trait	Symbol	Lahoma 2003	Stillwater 2003	Stillwater 2001	Altus 2002	Stillwater 2002	Lahoma 2002	Altus 2003	Total	Consistent linkage groups †
Yield traits										
Grain yield	GY	*2BL, 5A,* **1AL** 2BS	***4B***, ***5B,*** *6B, 7A,* **1B,** 7DL3	*2BL, 5A, 6A,* ***6B***, 3AS2, **4AL**	***5A***, *6A, 7A*	*4B, 5A, 5B, 6A,* ***7A***	***4B***, *5A, 6B*	***2BL***, ***5A***, ***7A***, 3BL, **7DL2**	**13**-19	***5A***
Spike number	SN			*1B*, **3BS**, 6A		*1B*			**1**-3	*1B*
Kernel number spike^{-1}	KS	*1AL,* ***2BS***, *4B,* ***6A***, 2BL, **2DL2**	*2BS, 4B,* ***6A***, 5A, 7A	*6A,* **1B,** 1AS **7BS2**, 7DL	*3BS, 4B,* ***6A***	***1AL***, ***3BS,*** ***4B***, *6A,* 2DL, 3BL			**10**-15	***6A***
Spike weight	SW	*6B,* **2BL**, **3BL2**	***1B***, 2BS	***6B,*** 1AL	***1B***, ***3BS,*** ***6B***, **5A**	***3BS***, **2DL**, 4AL, 6A			**10**-5	***6B***
Plant adaptation traits										
Heading date	HD	*3BL2,* 2BS, 7BL	*3A5A, 4B, 5B,* **6B**		*3A5A, 4B,* ***5B***	***3BL2***, *5B,* 3BS			**3**-10	***5B***
Maturity date	MD		***1B***, *5B,* **3AS2, 6B**	***1B***, *5B,* 7A					**4**-3	***1B,*** *5B*
Plant height	HT	***6A***	*2BL,* ***4B***, ***6A,***	***2BL***, ***4B***, **2DL**,		***2BS***, *3BL,*	***2BS***, ***3BL***, ***4B***,		**13**-6	***4B, 6A***

Trait	Symbol	Environments							Total	Consistent linkage groups †
		Lahoma 2003	Stillwater 2003	Stillwater 2001	Altus 2002	Stillwater 2002	Lahoma 2002	Altus 2003		
			5B	2DL2		***4B***, ***6A***	***6A***, 4AL, 6B			
Shattering score	SH	*3BL, 6B,* 3BL2	***6B***, **7DL**			*3BL,* ***4B****, 6B, 7A*	1AL, 2DL, **5A**	*4B,* ***7A****,* 2DL2, **6A**	**6**-10	***6B***
Lodging score	L	***5A***, **4AL**, 5B	***5A***, 3A5A, 4B			**1B**, 2DL, 6B, 7BS2			**4**-6	***5A***
Leaf yellowing	Y	***5A***, ***6A***, **2DL2, 7DL3**	***5A***, ***6A***, 1B, 4B			5B	***5A***, **2BL**, 2BS, **3BS**		**9**-4	***5A***
Leaf rust reaction	LR	4B, 7BS2				***1B****, 2DL2, 3BL,* ***3BS****, 6B*	*1B,* ***2DL2****, 3BL,* ***3BS****, 6B,* 2BS		**4**-9	***3BS***
Spike morphology										
Spike length	SL	***2BS****, 3BL, 5B,* **1AL**, 6A	***1AS***, ***1B****, 3BL2, 5B,* ***7A***	*2BS,* ***3BL****, 3BL2,* ***5B****,* **2BL**	***1AS****, 3BL,* ***5B***	*1B, 3BL, 7A,* 2DL, **4B**, **7BS**			**12**-12	***3BL***
Spike density	SD	*1B, 6B,* **4AL**	***1B****,* 4B, 5B, **7DL3**	*6B,* 2BL, **7BS**					**4**-6	***1B,*** *6B*
Awns	A	***7BS2***	***7BS2***	*3AS2,* 6A		*3AS2*			**2**-3	***7BS2***
Total		**17**-21	**22**-22	**14**-19	**8**-5	**16**-28	**12**-13	**6**-3	**95**-111	

† Linkage group(s) with the highest consistency across environments

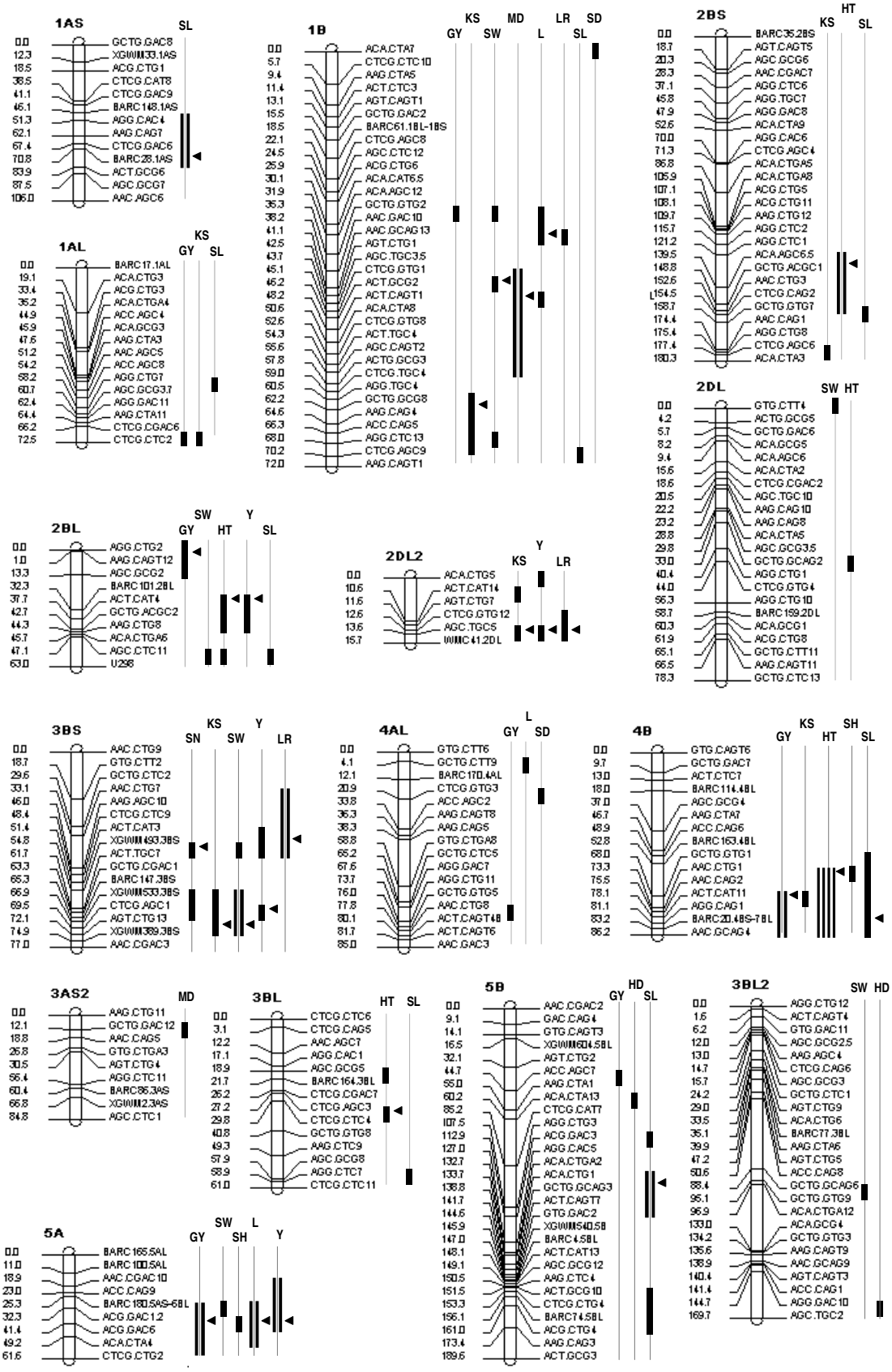
Table 3. Primary genomic regions and their associated additive gene effects for grain yield related traits, plant adaptation traits, and spike morphology identified by composite interval mapping (CIM) with a minimum LOD threshold of 3.0.

Linkage group	Position	Marker interval	LOD	a†	R^2
	cM				%
Grain yield				kg ha^{-1}	
1AL	66	*CTCG.CGAC6/CTCG.CTC2*	3.2	-252	9.4
1B	35	*GCTG.GTG2/AAC.GAC10*	3.4	-172	9.6
2BL	3	*AAG.CAGT12/AGC.GCG2*	3.5	253	11.3
4AL	78	*AAC.CTG8/ACT.CAGT4B*	3.2	181	7.6
4B	78	*ACT.CAT11/AGG.CAG1*	4.0	267	10.2
5A	38	*ACG.GAC1.2/ACG.GAC6*	6.0	241	18.5
5B	49	*ACC.AGC7/AAG.CTA1*	3.1	-185	11.2
6B	39	*GCTG.CTT1/GTG.GAC9*	3.1	175	7.3
7A	103	*BARC108.7AL-S/AGG.CAG10*	7.0	-361	21.1
7DL2	4	*BARC97.7DL/AAC.CGAC9*	3.3	-384	10.6
Spike number m^{-2}				no.	
3BS	59	*XGWM493.3BS/ACT.TGC7*	4.3	-25	12.0
Kernel number spike^{-1}				no.	
1AL	68	*CTCG.CGAC6/CTCG.CTC2*	3.6	1.2	9.0
1B	62	*GCTG.GCG8/AAG.CAG4*	5.1	1.9	12.0
2BS	179	*CTCG.AGC6/ACA.CTA3*	3.3	1.2	9.3
2DL2	14	*AGC.TGC5/WMC41.2DL*	4.5	-1.3	12.2
3BS	72	*AGT.CTG13/XGWM389.3BS*	3.9	1.2	8.7
4B	78	*ACT.CAT11/AGG.CAG1*	6.0	1.5	14.1
6A	80	*AAC.CTG5/AAC.CTG5*	7.4	-2.1	21.0
7BS2	24	*CTCG.CAT2/AGT.CTG3*	4.1	1.7	9.6
Spike weight				g	
1B	46	*ACT.GCG2/ACT.CAGT1*	3.5	-0.06	7.9
2BL	61	*AGC.CTC11/U298*	3.1	-0.04	11.0
2DL	0	*GTG.CTT4/ACTG.GCG5*	4.1	0.05	9.7
3BL2	88	*GCTG.GCAG6/GCTG.GTG9*	3.2	-0.04	9.9
3BS	72	*AGT.CTG13/XGWM389.3BS*	4.8	0.04	11.3
5A	25	*BARC180.5AS-6BL/ACG.GAC1.2*	4.7	0.06	10.7
6B	95	*AGC.TGC7/ACA.GCG1.2*	4.5	0.06	13.2

Linkage group	Position	Marker interval	LOD	a†	R^2
	cM				%
Heading date				d	
3BL2	169	*AGG.GAC10/AGC.TGC2*	3.3	-1.1	9.3
5B	60	*ACA.CTA13/CTCG.CAT7*	4.7	-1.1	12.0
6B	77	*AGG.CTC5/ACA.CTGA7*	3.4	-0.9	10.7
Maturity date rating				(1-4)	
1B	50	*ACT.CAGT1/ACA.CTA8*	3.9	-0.27	9.9
3AS2	14	*GCTG.GAC12/AAC.CAG5*	3.3	0.28	10.0
6B	57	*AAG.CTG5/DUPW216.6B*	4.2	-0.32	11.5
Plant height				cm	
2BL	40	*ACT.CAT4/GCTG.ACGC2*	6.0	3.0	16.7
2BS	144	*ACA.AGC6.5/GCTG.ACGC1*	6.0	-3.8	16.9
2DL	33	*GCTG.GCAG2/AGG.CTG1*	4.9	2.8	12.3
3BL	27	*CTCG.AGC3/CTCG.CTC4*	4.4	2.9	9.6
4B	75	*AAC.CTG1/AAC.CAG2*	6.7	-2.8	14.9
6A	87	*AGC.TGC4/ACC.AGC5*	5.6	2.5	12.1
Shattering score				(1-5)	
4B	73	*AAC.CTG1/AAC.CAG2*	3.5	-0.21	9.2
5A	32	*ACG.GAC1.2/ACG.GAC6*	3.3	-0.36	8.9
6A	63	*CTCG.GTG2/AAC.CGAC8*	3.2	0.50	9.3
6B	93	*ACA.CTG16/AGC.TGC7*	3.3	0.84	10.2
7A	99	*GCTG.GCG2/BARC108.7AL-S*	3.3	0.59	12.1
7DL	56	*AAC.AGC10/AAG.CTA8*	9.8	-0.61	55.9
Lodging score				(1-5)	
1B	41	*AAC.GCAG13/AGT.CTG1*	7.1	0.37	16.7
4AL	4	*GCTG.CTT9/BARC170.4AL*	5.0	0.36	14.1
5A	38	*ACG.GAC1.2/ACG.GAC6*	5.9	-0.39	23.0
Leaf yellowing				(1-5)	
2BL	38	*ACT.CAT4/GCTG.ACGC2*	4.6	-0.29	11.0
2DL2	16	*AGC.TGC5/WMC41.2DL*	5.2	0.31	14.5
3BS	72	*CTCG.AGC1/AGT.CTG13*	4.0	0.27	9.3
5A	38	*ACG.GAC1.2/ACG.GAC6*	6.0	-0.35	16.6

Linkage group	Position	Marker interval	LOD	a†	R^2
	cM				%
6A	31	*ACA.CTA1.5/AAC.GAC1*	4.3	-0.35	12.3
7DL3	29	*CTCG.GTG9/AAG.CTC6*	3.4	-0.26	8.7
Leaf rust reaction				%	
1B	41	*AAC.GCAG13/AGT.CTG1*	3.4	7.7	7.4
2DL2	16	*AGC.TGC5/WMC41.2DL*	3.5	-9.2	7.9
3BS	51	*ACT.CAT3/XGWM493.3BS*	7.2	-11.6	16.9
Spike length				cm	
1AL	58	*AGG.CTG7/AGC.GCG3.7*	4.1	-0.44	12.8
1AS	79	*BARC28.1AS/AGT.GCG6*	3.3	0.31	10.8
1B	70	*CTCG.AGC9/AAG.CAGT1*	3.7	-0.30	9.6
2BL	53	*AGC.CTC11/U298*	3.8	0.37	11.9
2BS	159	*GCTG.GTG7/AAC.CAG1*	4.3	0.31	13.7
3BL	61	*AGG.CTC7/CTCG.CTC11*	3.3	-0.30	7.4
4B	83	*BARC20.4BS-7BL/AAC.GCAG4*	8.2	0.40	18.0
5B	134	*ACA.CTG1/GCTG.GCAG3*	6.8	-0.44	16.6
7A	24	*CTCG.CAT1/AAG.AGC12*	4.7	0.40	17.1
7BS	39	*AGC.GCG13/AGG.CTC3*	4.3	-0.28	8.7
Spike density				(1- 4)	
1B	0	*ACA.CTA7/CTCG.CTC10*	3.2	-0.24	9.8
4AL	21	*CTCG.GTG3/ACC.AGC2*	3.5	0.22	11.8
7BS	38	*E13/AGC.GCG13*	5.8	-0.33	15.9
7DL3	7	*BARC172.7DL/GTG.CAGT4*	3.8	-0.29	14.1

† 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.


1AS
SL
0.0 GCTG.GAC8
12.3 XGWM33.1AS
18.5 ACG.CTG1
38.5 CTCG.CAT8
41.1 CTCG.GAC9
45.1 BARC148.1AS
51.3 AGG.CAC4
62.1 AAG.CAG7
67.4 CTCG.GAC6
70.8 BARC28.1AS
83.9 ACT.GCG6
87.5 AGC.GCG7
105.0 AAC.AGC6
1AL
KS
GY SL
0.0 BARC17.1AL
19.1 ACA.CTG3
33.4 ACG.CTG3
35.2 ACA.CTGA4
44.9 ACC.AGC4
45.9 ACA.GCG3
47.6 AAG.CTA3
51.2 AAC.AGC5
54.2 ACC.AGC8
58.2 AGG.CTG7
60.7 AGC.GCG3.7
62.4 AGG.GAC11
64.4 AAG.CTA11
66.2 CTCG.CGAC6
72.5 CTCG.CTC2
1B
KS MD LR SD
GY SW L SL
0.0 ACA.CTA7
5.7 CTCG.CTC10
9.4 AAG.CTA5
11.4 ACT.CTC3
13.1 AGT.CAGT1
15.5 GCTG.GAC2
18.5 BARC61.1BL-1BS
22.1 CTCG.AGC8
24.5 AGC.CTC12
25.9 ACG.CTG6
30.1 ACA.CAT6.5
31.9 ACA.AGC12
35.3 GCTG.GTG2
38.2 AAC.GAC10
41.1 AAC.GCAG13
42.5 AGT.CTG1
43.7 AGC.TGC3.5
45.1 CTCG.GTG1
46.2 ACT.GCG2
48.2 ACT.CAGT1
50.6 ACA.CTA8
52.6 CTCG.GTG8
54.3 ACT.TGC4
55.6 AGC.CAGT2
57.8 ACTG.GCG3
59.0 CTCG.TGC4
60.5 AGG.TGC4
62.2 GCTG.GCG8
64.6 AAG.CAG4
66.3 ACC.CAG5
68.0 AGG.CTC13
70.2 CTCG.AGC9
72.0 AAG.CAGT1
2BS
HT
KS SL
0.0 BARC35.2BS
18.7 AGT.CAGT5
20.3 AGC.GCG6
28.3 AAC.CGAC7
37.1 AGG.CTC6
45.8 AGG.TGC7
47.9 AGG.GAC8
52.6 ACA.CTA9
70.0 AGG.CAC6
71.3 CTCG.AGC4
86.8 ACA.CTGA5
105.9 ACA.CTGA8
107.1 ACG.CTG5
108.1 ACG.CTG11
109.7 AAG.CTG12
115.7 AGG.CTC2
121.2 AGG.CTC1
139.5 ACA.AGC6.5
148.8 GCTG.ACGC1
152.6 AAC.CTG3
154.5 CTCG.CAG2
158.7 GCTG.GTG7
174.4 AAC.CAG1
175.4 AGG.CTG8
177.4 CTCG.AGC6
180.3 ACA.CTA3
2DL
SW HT
0.0 GTG.CTT4
4.2 ACTG.GCG5
5.7 GCTG.GAC6
8.2 ACA.GCG5
9.4 ACA.AGC6
15.6 ACA.CTA2
18.6 CTCG.CGAC2
20.5 AGC.TGC10
22.2 AAG.CAG10
23.2 AAG.CAG8
28.8 ACA.CTA5
29.8 AGC.GCG3.5
33.0 GCTG.GCAG2
40.4 AGG.CTG1
44.0 CTCG.GTG4
56.3 AGG.CTG10
58.7 BARC159.2DL
60.3 ACA.GCG1
61.9 ACG.CTG8
65.1 GCTG.CTT11
66.5 AAG.CAGT11
78.3 GCTG.CTC13
2BL
SW Y
GY HT SL
0.0 AGG.CTG2
1.0 AAG.CAGT12
13.3 AGC.GCG2
32.3 BARC101.2BL
37.7 ACT.CAT4
42.7 GCTG.ACGC2
44.3 AAG.CTG8
45.7 ACA.CTGA6
47.1 AGC.CTC11
63.0 U298
2DL2
Y
KS LR
0.0 ACA.CTG5
10.6 ACT.CAT14
11.6 AGT.CTG7
12.6 CTCG.GTG12
13.6 AGC.TGC5
15.7 WMC41.2DL
3BS
KS Y
SN SW LR
0.0 AAC.CTG9
18.7 GTG.CTT2
29.6 GCTG.CTC2
33.1 AAC.CTG7
46.0 AAG.AGC10
48.4 CTCG.CTC9
51.4 ACT.CAT3
54.8 XGWM493.3BS
61.7 ACT.TGC7
63.3 GCTG.CGAC1
65.3 BARC147.3BS
66.9 XGWM533.3BS
69.5 CTCG.AGC1
72.1 AGT.CTG13
74.9 XGWM369.3BS
77.0 AAC.CGAC3
4AL
L
GY SD
0.0 GTG.CTT6
4.1 GCTG.CTT9
12.1 BARC170.4AL
20.9 CTCG.GTG3
33.8 ACC.AGC2
36.3 AAG.CAGT8
38.3 AAG.CAG5
53.8 GTG.CTGA8
65.2 GCTG.CTC5
67.6 AGG.GAC7
73.7 AGG.CTG11
76.0 GCTG.GTG5
77.8 AAC.CTG8
80.1 ACT.CAGT4.8
81.7 ACT.CAGT6
85.0 AAC.GAC3
4B
KS SH
GY HT SL
0.0 GTG.CAGT6
9.7 GCTG.GAC7
13.0 ACT.CTC7
18.0 BARC114.4BL
37.0 AGC.GCG4
45.7 AAG.CTA7
48.9 ACC.CAG6
52.8 BARC163.4BL
68.0 GCTG.GTG1
73.3 AAC.CTG1
75.5 AAC.CAG2
78.1 ACT.CAT11
81.1 AGG.CAG1
83.2 BARC20.4BS-7BL
86.2 AAC.GCAG4
3AS2
MD
0.0 AAG.CTG11
12.1 GCTG.GAC12
18.8 AAC.CAG5
26.8 GTG.CTGA3
30.5 AGT.CTG4
56.4 AGG.CTC11
60.4 BARC86.3AS
66.8 XGWM2.3AS
84.8 AGC.CTC1
3BL
HT SL
0.0 CTCG.CTC6
3.1 CTCG.CAG5
12.2 AAC.AGC7
17.1 AGG.CAC1
18.9 AGC.GCG5
21.7 BARC164.3BL
25.2 CTCG.CGAC7
27.2 CTCG.AGC3
29.8 CTCG.CTC4
40.8 GCTG.GTG8
49.3 AAG.CTC9
57.9 AGC.GCG8
59.9 AGG.CTC7
61.0 CTCG.CTC11
5B
GY HD SL
0.0 AAC.CGAC2
9.1 GAC.CAG4
14.1 GTG.CAGT3
16.5 XGWM604.5BL
32.1 AGT.CTG2
44.7 ACC.AGC7
55.0 AAG.CTA1
60.2 ACA.CTA13
85.2 CTCG.CAT7
107.5 AGG.CTG3
112.9 ACG.GAC3
127.0 AGG.CAC5
132.7 ACA.CTGA2
133.7 ACA.CTG1
136.8 GCTG.GCAG3
141.7 ACT.CAGT7
144.6 GTG.GAC2
145.9 XGWM540.5B
147.0 BARC4.5BL
148.1 ACT.CAT13
149.1 AGC.GCG12
150.5 AAG.CTC4
151.5 ACT.GCG10
153.3 CTCG.CTG4
156.1 BARC74.5BL
161.0 ACG.CTG4
173.4 AAG.CAG3
189.6 ACT.GCG3
3BL2
SW HD
0.0 AGG.CTG12
1.6 ACT.CAGT4
6.2 GTG.GAC11
12.0 AGC.GCG2.5
13.0 AAG.AGC4
14.7 CTCG.CAG6
15.7 AGC.GCG3
24.2 GCTG.CTC1
29.0 AGT.CTG9
33.5 ACA.CTG6
36.1 BARC77.3BL
39.9 AAG.CTA6
47.2 AGT.CTG5
50.6 ACC.CAG8
88.4 GCTG.GCAG6
95.1 GCTG.GTG9
96.9 ACA.CTGA12
133.0 ACA.GCG4
134.2 GCTG.GTG3
135.6 AAG.CAGT9
138.9 AAC.GCAG9
140.4 AGT.CAGT3
141.4 ACC.CAG1
144.7 AGG.GAC10
169.7 AGC.TGC2
5A
SW L
GY SH Y
0.0 BARC165.5AL
11.0 BARC100.5AL
18.9 AAC.CGAC10
23.0 ACC.CAG9
25.3 BARC180.5AS-6BL
32.3 ACG.GAC1.2
41.4 ACG.GAC6
49.2 ACA.CTA4
61.6 CTCG.CTG2

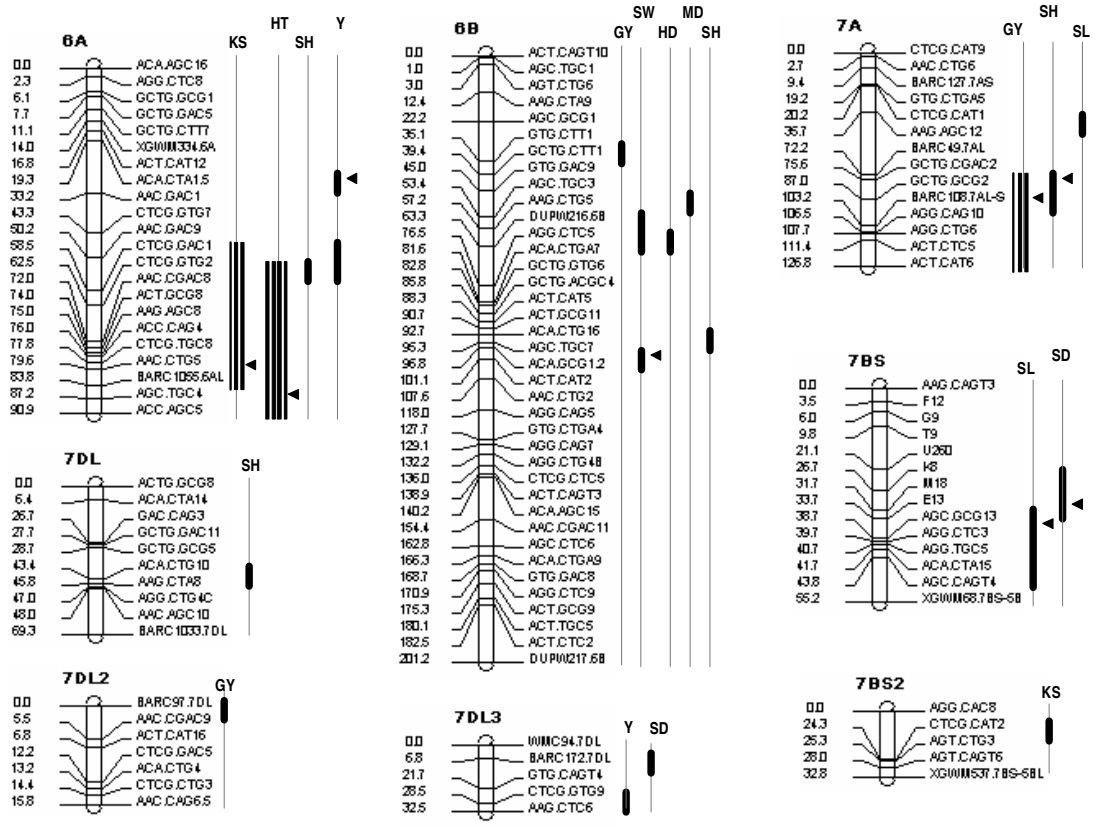


Fig. 1 Primary genomic regions of major QTLs (LOD > 3) identified by composite interval mapping for grain yield and yield components, plant adaptation traits, and spike morphology from the Ning7840 x Clark RIL population evaluated in Oklahoma from 2001 to 2003. Bars indicate the number of environments for which the same marker interval was detected. Triangles indicate the interval exhibiting the peak LOD value.

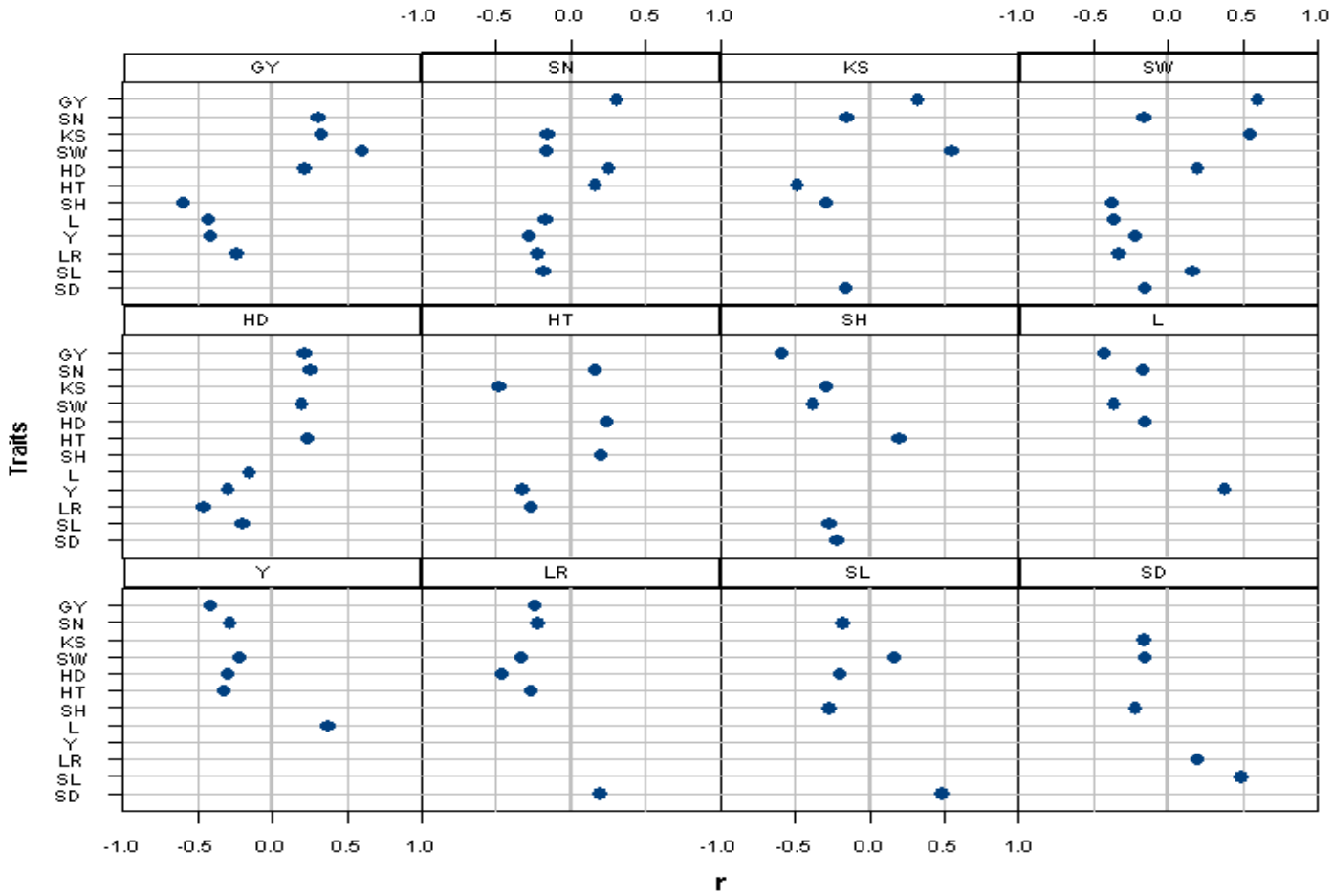


Fig. 2 Correlation coefficient plot among yield related traits, plant adaptation traits, and spike morphology for the Ning7840 x Clark RIL population evaluated in Oklahoma from 2001 to 2003. Only significant *r*-values ($P < 0.05$) are shown in the plot. Traits are grain yield (GY), spike number (SN), kernel number spike^{-1} (KS), spike weight (SW), heading date (HD), plant height (HT), shattering score (SH), lodging score (L), leaf yellowing (Y), leaf rust reaction (LR), spike length (SL), and spike density (SD).

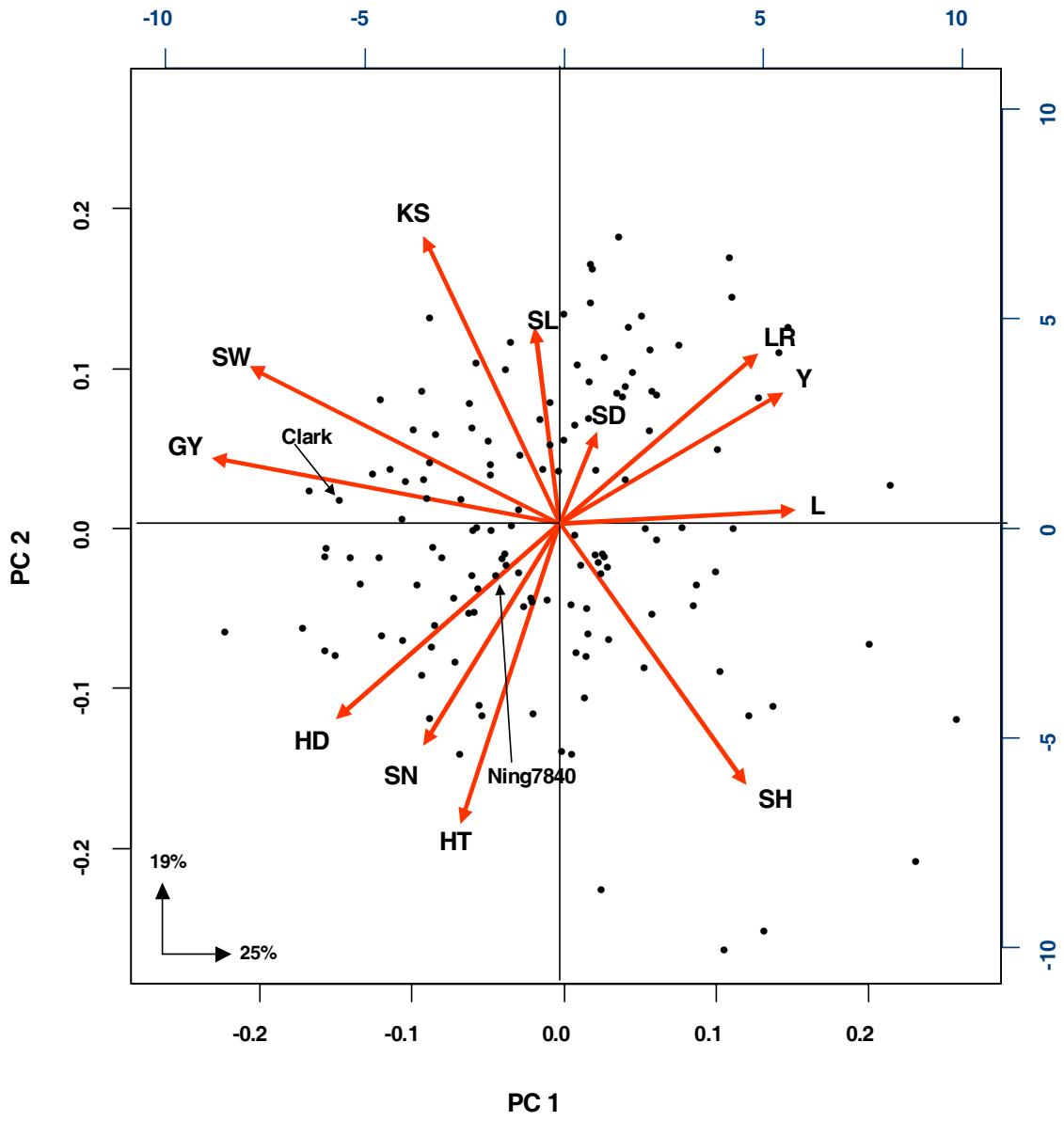


Fig. 3 Principal component (PC) analysis biplot summarizing the relationship among yield traits, plant adaptation traits, and spike morphology for the Ning7840 x Clark RIL population evaluated in Oklahoma from 2001 to 2003. Traits are grain yield (GY), spike number (SN), kernel number spike^{-1} (KS), spike weight (SW), heading date (HD), plant height (HT), shattering score (SH), lodging score (L), leaf yellowing (Y), leaf rust reaction (LR), spike length (SL), and spike density (SD).

APPENDIX

Table 1. Location and years for which traits pertaining to grain yield, plant adaptation, and spike morphology were measured (X) in the Ning7840 x Clark RIL population (Stillwater, ST; Lahoma, LA; and Altus, AL, Oklahoma).

Trait category	Trait Abbreviation	2001	2002			2003		
		ST	ST	LA	AL	ST	LA	AL
Yield								
Grain yield	GY	X	X	X	X	X	X	X
Spike number	SN	X	X					
Kernel number spike^{-1}	KS	X	X		X	X	X	
Spike weight	SW	X	X		X	X	X	
Plant adaptation								
Heading date	HD		X	X		X	X	
Maturity date	MD	X				X		
Plant height	HT	X	X	X		X	X	
Shattering score	SH		X	X		X	X	X
Lodging score	L		X			X	X	
Leaf yellowing	Y		X	X		X	X	
Leaf rust reaction	LR		X	X			X	
Spike morphology								
Length	SL	X	X		X	X	X	
Density	SD	X				X	X	
Awns	A	X	X			X	X	

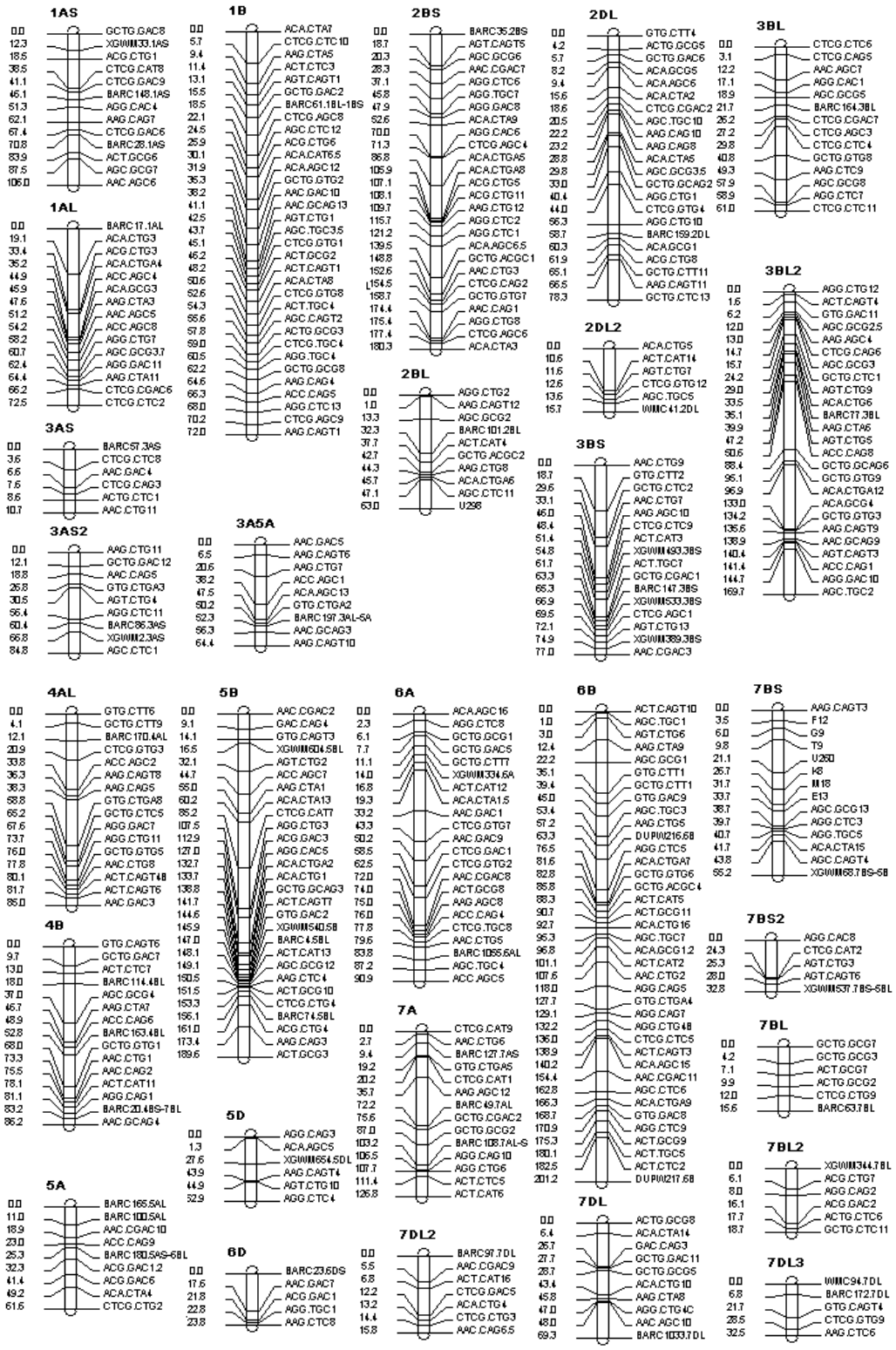


Fig. 1. Integrated AFLP and SSR linkage map based on Ning7840 x Clark RIL population. Cumulative distances between markers are given in cM, calculated from recombination frequencies according to Kosambi mapping function.

CHAPTER II

MAPPING QUANTITATIVE TRAIT LOCI FOR QUALITY FACTORS IN AN INTER-CLASS POPULATION OF U.S. AND CHINESE WHEAT

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ABSTRACT

Grain quality factors are important in determining the suitability of wheat (*Triticum aestivum* L.) for end-use product value, and they constitute prime targets for marker-assisted selection. The objective of this study was to identify quantitative trait loci (QTLs) that influence 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 facultative wheat Ning7840 and the soft red winter (SRW) wheat Clark. The population was grown at three Oklahoma locations from 2001 to 2003. In addition to wheat protein, physical factors such as test weight, kernel weight, and kernel diameter, and class factors such as hardness index, were characterized. The map of this population consisted of 410 markers (363 AFLP and 47 SSR) in 29 linkage groups. The additive effects of individual QTLs identified by composite interval mapping analysis accounted for up to 27% of the phenotypic variation. Positive phenotypic correlations were found among physical factors. A unique QTL was identified for test weight in linkage group 5B that influenced test weight independent of kernel weight and presumably through grain packing efficiency. Common markers were identified for test weight, kernel weight, and kernel diameter on 5A. Consistent co-localized QTLs were identified for kernel weight and kernel diameter in linkage group 6A. Unique consistent genomic regions on 1B and on 1AL were associated with kernel weight and kernel diameter, respectively. Consistent QTLs were also identified with specific effects for hardness index (3AS2 and 7BS2) and wheat protein (2BL, 4B, 6B, and 7BL). The consistency of physical factor QTLs across environments reveals their potential for marker-assisted selection.

INTRODUCTION

The economic value of wheat (*Triticum aestivum* L.) is framed by intrinsic quality factors that affect the end-use product (Morris and Rose, 1996; Ammiraju et al., 2001). Physical factors, described by test weight, kernel weight, and kernel size, determine milling yield if not agronomic yield (Varshney et al., 2000; Dholakia et al., 2003). Wheat class factors, described by kernel hardness and protein content, 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 et al., 2002).

As a result of genetic analysis using classical and aneuploid methods, several hundred wheat genes have been identified, but for only a few have their function and effects been described. Among them, market class differences in kernel hardness can be explained by allelic differences at a single locus, *Ha,* on chromosome 5D, identified through a marker protein for kernel softness called friabilin containing two major polypeptides, puroindolines *a* and *b* (Nelson et al., 1995; Martin et al., 2001; Lillemo et al., 2002). Though extensively studied, grain protein content has proven to be one of the more difficult traits to genotype. To date, only four genes have been identified: *pro*1 and *pro*2 on chromosome 5D and 5A, and unnamed genes on 2D (Prasad et al., 1999) and 6B (Khan et al., 2000; Distelfeld et al., 2003). All genes have been recognized as quantitative trait loci (QTL), and no major genes have been discovered. In addition to its direct effect on baking quality, Galande et al. (2001) suggest that protein content may have indirect effects on kernel weight and test weight.

Earlier studies on physical factors reported that test weight is influenced by kernel shape, uniformity, density, and kernel packing efficiency (Campbell et al., 1999; Galande

et al., 2001). Kernel weight and size are controlled by several QTLs located on as many as 15 chromosomes (Campbell et al., 1999; Galande et al., 2001; Dholakia et al. 2003). Unfortunately, genetic improvement in kernel weight may be compromised by a concomitant reduction in kernel number per spike, thus neutralizing the agronomic benefit derived from increased kernel weight (Marshall et al., 1984; Wiersma et al., 2001). However, relatively small increases in kernel weight or kernel size, at the same yield level, should have a proportionately favorable impact on milling quality.

Molecular markers have provided a useful tool for a clearer understanding of the genetic basis of important traits in a variety of crops. Two marker systems have been frequently used to characterize species with relatively large genome size such as wheat ($2n = 6x = 42$, 16,000 Mbp): simple sequence repeat (SSR) and amplified fragment length polymorphism (AFLP). The former is evenly distributed across the genome, inherited in a co-dominant manner, chromosome specific, and an ideal marker system for map construction and marker-assisted selection (Röder et al., 2002). The AFLP is a multiplex marker system based on selective amplification of a limited number of DNA restriction fragments and has the advantage of permitting simultaneous coverage of several loci in a single assay (Vos et al., 1995).

The objective of this study was to identify and locate QTLs affecting wheat quality factors in a winter wheat population previously characterized for agronomic traits by Marza et al. (2005). Parental differences in kernel weight and hardness suggested this population could expand our understanding of the genetic control of milling quality. Hence our study focused on physical and market class components relating to kernel size and texture, test weight, and wheat protein content.

MATERIALS AND METHODS

Genetic material and experimental design

A population of 132 F_{12} recombinant inbred lines (RIL) was derived by single-seed descent from the F_2 of the cross, Ning7840/Clark (Bai et al., 1999). Ning7840 (Aurora/Anhui 11//Sumai 3) is a hard red facultative cultivar from China with type II scab resistance (Zhou et al., 2003) and relatively low yield potential. Clark is a SRW cultivar from Purdue University, Indiana (Ohm et al., 1988) with an early date of heading, relatively high yield potential, and high kernel weight. The RILs along with the parental genotypes were grown at three Oklahoma locations (Stillwater, Lahoma, and Altus) for three years 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

Information was collected on wheat quality factors relevant to this mapping population (Table 1). Test weight (TW) was measured in kg hL^{-1} from the weight of grain filling a 0.95-L container. The single-kernel-characterization system (SKCS) (Model 4100, Perten Instruments North America, Inc., Springfield, IL) was used to estimate kernel weight (KW, mg), kernel diameter (KD, mm), and SKCS-hardness index (HI-SK, on a scale of 0 = extremely soft to 100 = extremely hard) from a sample of 300 sound kernels per plot. Wheat protein content (WP, g kg^{-1}) and another assessment of hardness index (HI, same 0-to-100 scale) were determined by near-infrared reflectance (NIR) spectroscopy according to AACC method 39-70a (AACC, 1995) using 9 g ground, whole-wheat samples from each plot. Trait measurements were taken from at least five environments per trait (Table 1).

Isolation and amplification of DNA

Genomic DNA extraction from both parents and the 132 F_{12} RILs was carried out according to the cetylmethylammonium bromide (CTAB) method (Saghai-Maroof et al., 1984). Parental polymorphism was assessed with 400 SSR primers. The polymerase chain reaction (PCR) amplifications of microsatellite primers were performed in 12-µL reaction volumes in a thermal cycler (Perkin Elmer, Norwalk, CT). Amplified products were resolved by automated PCR product amplification with the Li-Cor IR-4200 DNA sequencer (Li-Cor Inc., Lincoln, NE) using a fluorescent-labeled M-13 primer for PCR detection, followed by SSR product separation in a 6.5% polyacrylamide gel in the Li-Cor IR-4200 DNA sequencer. 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

For constructing 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 missing (-). Linkage analysis was performed using the MAPMAKER program (Macintosh V2.0, Lander et al., 1987). Recombination frequencies were converted to centimorgans (cM) using the Kosambi mapping function (Kosambi, 1944).

Statistical analysis

Skewness and kurtosis were estimated to describe the phenotypic distributions relative to normality. The complete set of data from each environment was subjected to analysis of variance (ANOVA) to determine the effects of genotype (RIL and parent) and environment. Phenotypic correlations were calculated for all combinations of traits based

on RIL means across environments. Principal component (PC) analysis of genotypes across environments was performed based on standardized ($\mu = 0$, $\sigma = 1$) means data using PRINCOMP procedure of SAS (SAS Institute, 2003). Briefly, the resulting 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^o and 180^o angles indicating strong correlations and 90^o angles representing a weak correlation (Yan and Kang, 2003).

QTL analysis

A Windows version of QTL Cartographer V2.0 (Wang et al., 2004) was used to perform composite interval mapping (CIM) analysis based on model 6 of the Zmapqtl procedure (Basten et al., 2001). The closest marker to each local LOD peak was used as a cofactor. The walking speed for scanning the genome was set at 2.0 cM. The LOD threshold used to declare a significant QTL was estimated from 1000 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) using single-factor analysis from a general linear model procedure (Basten et al., 2001). For each QTL, R^2 was determined for the single marker closest to the identified QTL.

RESULTS AND DISCUSSION

Linkage map

The map for this population included 410 markers (363 AFLP and 47 SSR) distributed across 29 linkage groups of five or more markers. Each linkage group contained at least one SSR marker. Total map distance spanned 2,223 cM with a mean interval length of 5.4 cM. Linkage groups were designated by chromosome number, and chromosome arm if known. Most of the markers (93%) fit the expected 1:1 segregation ratio for F_{12} RIL. Therefore, the saturated map fulfilled basic requirements to perform a whole-genome QTL scan.

Phenotypic summary

Between the parents, Clark produced heavier kernels (29.7 mg KW) and larger kernels (2.26 mm kernel diam) across environments ($P < 0.05$) compared to Ning7840 (26.3 mg KW and 2.14 mm kernel diam) (Table 2). As expected for a SRW wheat, Clark produced lower values for both measurements of hardness index. Despite these differences in kernel size and texture, both parents produced similar values for test weight and wheat protein content.

Most values for skewness and kurtosis did not exceed 1.0 (Table 2), indicating the RIL phenotypic distributions exhibited normality except for hardness index (Fig. 1). The RILs apparently segregated for few genes with major effects on hardness, as indicated by the bimodal distributions for NIR and SKCS measurements. That transgressive segregation occurred in both directions for all traits implies that a high level of gene dispersion existed between the parents of this population. In general, all traits exhibited polygenic segregation patterns and continuous variation.

Positive correlations were observed between test weight and kernel weight or kernel diameter (Fig. 2). Hence RILs with higher test weight tended to have larger, heavier kernels. Previous studies in bread wheat on correlation of these factors varied from positive (Gibson et al., 1998) to slightly negative (Schuler et al., 1994). Yamazaki and Briggle (1969) and Marshall et al. (1984) described the components of test weight as kernel weight (influenced by the density of the grain) and kernel morphology (affecting kernel packing efficiency). Differences in kernel morphology may modify the association of volumetric grain weight and kernel weight. Kernel weight and kernel diameter were also moderately associated with wheat protein content (Fig. 2).

The bi-trait correlations summarized in Fig. 2 may be extended to view multi-trait relationships within the space of RIL variation using the PC-biplot (Fig. 3). This biplot revealed two important genotype x trait trends: a strong association of PC1 with kernel size factors (kernel diameter and kernel weight), and the separation of two distinctive clusters of genotypes by PC2 according to hardness index. Kernel diameter and kernel weight showed a strong association in the biplot, as did test weight and kernel diameter. Protein content showed close association with kernel weight, but the relatively short vector for wheat protein (or relatively low differentiation among RILs for wheat protein) compromises the significance of their association.

Earlier reports indicated that kernel hardness index and wheat protein content were positively correlated, in which hard wheat was generally higher in protein content than soft wheat (Bushuk, 1998). However, no association was found in our population across all RILs with major and minor differences in hardness index (Fig. 3). When the RILs were grouped on the basis of relatively high HI (> 40 HI-SK, $n = 64$) and low HI (≤ 40

HI-SK, $n = 68$), mean wheat protein content of the hard RILs across environments was only 2 g kg^{-1} or 0.2 percentage units greater ($P > 0.05$) than that of the soft RILs. In contrast, wide variation ($P < 0.05$) observed for wheat protein within each hardness group. Within groups, the harder RILs showed a significant correlation for HI-SK vs wheat protein ($r = 0.42$, $P < 0.01$), which is consistent with Carver (1994), while no significant correlation was detected within the softer RILs. Any QTL that might be associated with wheat protein content in this population is therefore not expected to represent a pleiotropic effect of major genes conferring hardness differences.

QTL mapping

Summarizing the molecular linkage map and composite interval-mapping analysis, we detected a total of 131 putative major and minor QTLs. Among all quality traits, the highest frequency of QTLs was found in the B genome with 70 QTLs (53%); 46 (35%) QTLs were found in the A genome and 15 (12%) in the D genome. Most of the QTLs identified for kernel weight and kernel diameter were associated with genomes A and B, whereas QTLs for test weight, protein content, NIR-hardness index, and SKCS-kernel hardness were associated with genome B (Table 3). All quality traits here showed a weak association with D genome. The number of QTLs from homoeologous groups one to seven were 7 (5%), 13 (10%), 23 (18%), 16 (12%), 31 (24%), 20 (15%), and 21 (16%), respectively. Chromosomes 2A, 3D, and 4D were not included in the analysis. The mean number of putative QTLs detected in this study was five for test weight and kernel size and four for wheat protein and hardness.

Test weight (TW)

Markers associated with test weight were concentrated in linkage groups 4B, 5A, 5B, and 6B (Table 4). The phenotypic contributions of an individual linkage group

ranged from 9 to 21%. The QTLs in linkage groups 5A and 5B were the most consistently detected in four and five of the seven environments, respectively. Markers in linkage group 5B were exclusively associated with test weight, where AFLP marker interval *ACT.CAGT7/GTG.GAC2* was the most common across environments. The Clark allele from the identified region on 5A increased test weight, while on 5B the Ning7840 allele increased test weight. Several of the markers associated with test weight on 5A were also associated with kernel weight and kernel diameter (Table 4), as may be expected from the high phenotypic correlation among these traits. Moreover, the marker interval *BARC180/ACA.CTA4* was consistently identified as common for all traits (Fig. 4).

Contrary to the similar test weights of the parents across environments (mean difference of 0.4 kg hL^{-1}), their kernel morphology differed noticeably. Kernels of Ning7840 were narrow and long, whereas kernels of Clark were short and rounded (plump). The QTL on 5B may influence one component of test weight, packing efficiency, through its effect on kernel morphology, since that was the only distinctive contribution of Ning7840 to higher test weight, at least with respect to linkage group 5B. To test that hypothesis, we classified the RILs based on the most consistent marker interval on 5B (*ACT.CAGT7/GTG.GAC2*), with or without the purported allele from Ning7840. Using kernel characteristics based on Briggle and Reitz (1963), kernels of RILs with the Ning7840 allele exhibited a crease with narrow width and shallow depth, angular cheeks, and a tendency toward oval shape. On the other hand, kernels of RILs without the Ning7840 allele had midwide and middeep crease, rounded cheeks, and tendency toward ovate shape. These patterns were consistent across all environments in which kernel samples were available (5 of 7 environments). To further support these

visual observations, test weight was compared between marker groups. The RILs with the Ning7840 allele exceeded those without by 1.08 kg hL^{-1} ($P < 0.05$). Interestingly, those same groups differed by only 0.02 mm kernel diameter. Differences in kernel weight were negligible.

Our QTL analysis not only accounted for test weight variation through the interval relating to packing efficiency in linkage group 5B but also through the interval in linkage group 5A (*BARC180/ACA.CTA4*) relating to kernel weight and kernel diameter. To our knowledge, there are very few molecular mapping studies which target test weight. The two minor QTLs on 2BS and 4AL, along with the major QTL on 5A, were coincident with QTLs reported by Campbell et al. (1999). Additionally, the QTL identified in linkage group 6B corroborates previous evidence of QTLs found in similar chromosome regions by Galande et al. (2001) and Elouafi et al. (2004).

Kernel weight and kernel diameter

Phenotypic variation for kernel weight and kernel diameter were highly informative in this population, evidenced by the relatively long trait vectors in the biplot (Fig. 3). For kernel weight, we identified major QTL regions in linkage groups 1B, 2BS, 3BS, 4B, 5A, 5D, 6A, and 6B (Table 4). These QTLs explained from 7 to 27% of the phenotypic variance. The most consistent QTLs for kernel weight were in linkage groups 5A and 6A, with their respective intervals, *BARC180/ACA.CTA4* and *AAC.GAC1/AAC.CGAC8*. The Clark allele for the majority of major QTLs listed above increased kernel weight. Lately, several attempts have been made to understand the genetic basis of kernel weight. Chromosome regions associated with kernel weight on 5AL were reported by Campbell et al. (1999); on 2B, 4B, 6B by Varshney et al. (2000) and Elouafi et al. (2004); on 6B by Ammiraju et al. (2001); and on 2B by Gross et al. (2003). Co-localization of QTLs was

observed between kernel weight and grain yield (Marza et al., 2005) in linkage groups 4B (*AGG.CAG1/AAC.GCAG4*) and 5A (*BARC180/ACA.CTA4*). This has important implications for simultaneous improvement of milling yield and grain yield (Marshall et al., 1984; Schuler et al., 1994).

Common QTL regions were identified for kernel weight and kernel diameter from several linkage groups (e.g., 4B, 5A, 5D, 6A, and 6B), as would be expected with their strong phenotypic relationship (Fig. 3). Among these, the major QTLs on 5A and 6A had the largest influence. The major QTL found on 5D for kernel weight and kernel diameter was the only QTL detected in that linkage group. A locus on 1B was exclusive to kernel weight, and though only identified in certain environments, QTLs on 2BS and 3BS also were uniquely associated with kernel weight.

Putative QTLs associated with kernel diameter were detected in linkage groups 1AL, 4B, 5A, 5D, 6A, 6B, and 7DL (Table 4 and Fig. 4). The Clark allele increased kernel diameter for most of those. The QTL regions on 5A and 6A were the most consistent across environments. Markers in linkage group 1AL, which were relatively consistent across environments, and those in 7DL identified from a single environment (ST01), were uniquely associated with kernel diameter. Our findings coincided with earlier reported QTLs on 5A (Campbell et al., 1999) and with a gene controlling kernel width on 1A (Gura and Saulescu, 1996), but none of the QTLs reported by Dholakia et al. (2003) on 2BL and 2DL were identified here.

Wheat protein content

Even with no difference in mean protein content of Clark and Ning7840 (136 g kg^{-1}), the RILs varied significantly from 123 to 157 g kg^{-1} (Table 2 and Fig. 1). With this level of transgressive segregation, four major consistent QTLs were detected for protein

content in linkage groups 2BL, 4B, 6B, and 7BL (Tables 3). They explained 9 to 13% of the phenotypic variance. Alleles from Clark showed positive effects on protein content on 6B and 7BL, and negative effects at the other QTLs. The QTLs on 4B were common to kernel weight and kernel diameter. Additionally, a QTL on 7BL was common to a minor QTL identified for kernel weight and hardness index (Table 3). One of the most widely studied quality traits in wheat is protein content. Prasad et al. (1999) and Campbell et al. (2001) reported QTLs for protein content on chromosomes 2B and 2D; however, the most widely reported QTLs were on 5D, 5A, and 6B (Khan et al., 2000; Olmos et al., 2003; Distelfeld et al., 2003).

NIR-hardness index and SKCS-hardness index

The bimodal distributions observed for both measurements of hardness index (Fig. 1) indicates that this population of RILs contained two distinct hardness classes, based either on differential particle size (NIR) of uniformly ground whole-wheat samples or on resistance to crushing (SKCS). Though hardness class differences can be attributed to allelic differences at single locus (Giroux et al., 1998), our study identified four genomic regions associated with NIR-hardness index on linkage groups 2DL, 3AS2, 5B, and 7BS2 (Table 4). Each region explained 10 to 18% of the phenotypic variance, and the allele from the soft wheat parent, Clark, decreased NIR-hardness index in all regions except one (2DL). Five QTLs in linkage groups 3AS2, 3BS, 4B, 7BS2, and 7DL2 were identified for SKCS-hardness index, explaining 10 to 15% of the phenotypic variance, and the allele from Clark decreased SKCS-hardness index in all QTLs except one (7DL2) (Table 4).

Puroindoline proteins *a* and *b* represent the molecular genetic basis of hardness variation attributable to chromosome 5DS (Morris, 2002). Our study was unable to

attribute any effect for kernel hardness to QTLs on that chromosome arm. Further marker screening with emphasis on chromosome 5D may be needed to identify marker associations in that critical region. Nevertheless, our study did find highly consistent QTLs for both methods of hardness estimation on linkage group 3AS2, which coincides with a previously reported QTL on the same arm (Campbell et al., 1999). Sourdille et al. (1996) reported minor effects for hardness on 2A, 2D, 5B and 6D. Isolated major QTLs identified here on 2DL and 5B may be related. The single common region associated with hardness index and protein content was a QTL region on 7BS2.

CONCLUSIONS

In this inter-class cross of U.S. and Chinese wheat, QTLs associated with test weight and kernel size were reduced to five genomic regions. A unique QTL in linkage group 5B (*ACT.CAGT7/GTG.GAC2*) was identified for test weight that indirectly appears related more to packing efficiency than kernel size. We identified another consistent major QTL for test weight in linkage group 5A (*ACG.GAC6/ACA.CTA4*) that appears pleiotropic to kernel weight and diameter and, thus, could impact kernel density rather than packing. The strong relationship between kernel weight and diameter was also reflected in the common QTL on linkage group 6A (*CTCG.GTG2/AAC.CGAC8*). Unique QTLs for kernel weight (1B) and kernel diameter (1AL) also were identified. We identified QTLs with specific effects for hardness index (3AS2 and 7BS2) and for wheat protein (2BL, 4B, 6B, and 7BL). Because end-use quality has multiple components that add complexity to breeding efforts, important common QTLs influencing more than one trait add value to an already valuable selection tool.

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Table 1. Locations and years for which traits pertaining to wheat quality factors were measured in the RIL population, Ning7840 x Clark (Stillwater, ST; Lahoma, LA; and Altus, AL, Oklahoma).

Trait	Symbol	2001	2002			2003		
		ST	ST	LA	AL	ST	LA	AL
Physical factor								
Test weight	TW	X	X	X	X	X	X	X
Kernel weight	KW	X	X		X	X	X	
Kernel diameter	KD	X	X		X	X	X	
Class factor								
Wheat protein	WP	X	X		X	X	X	
NIR-hardness index	HI	X	X		X	X	X	
SKCS-hardness index	HI-SK	X	X		X	X	X	

Table 2. Summary of phenotypic data for wheat quality factors of Ning7840, Clark, and their RIL progeny evaluated in various Oklahoma environments from 2001 to 2003.

Env.	Parents		RIL population†					
	Clark	Ning7840	Mean	Max	Min	SD	Skewness	Kurtosis
	Test weight, kg hL^{-1}							
ST01	70.9	69.6	70.9	76.0	61.9	2.3	-0.92	2.00
ST02	68.3	68.3	66.1	72.2	54.6	3.1	-0.87	1.21
LA02	69.2	69.6	67.5	72.6	59.3	2.5	-0.72	0.65
AL02	70.9	70.9	69.1	76.0	61.9	2.4	-0.34	0.43
ST03	70.6	70.3	70.1	74.8	64.0	2.1	-0.40	0.20
LA03	71.6	72.0	71.7	75.6	64.5	2.4	-0.87	0.63
AL03	72.0	70.3	70.8	74.8	64.9	1.9	-0.30	-0.24
	Kernel weight, mg							
ST01	32.4	24.8	29.8	37.6	21.6	3.3	-0.03	-0.38
ST02	28.7	25.5	25.2	32.2	19.2	2.8	0.11	-0.40
AL02	29.6	24.3	25.8	32.8	19.5	3.0	-0.02	-0.63
ST03	30.8	29.4	28.6	33.7	22.2	2.6	-0.12	-0.27
LA03	27.2	27.4	28.9	34.0	23.9	1.8	-0.20	0.21
	Kernel diameter, mm							
ST01	2.30	2.10	2.26	2.65	1.90	0.16	0.20	-0.50
ST02	2.23	2.13	2.10	2.57	1.80	0.16	0.25	0.03
AL02	2.27	2.03	2.06	2.47	1.73	0.15	-0.03	-0.52
ST03	2.31	2.26	2.23	2.50	1.93	0.13	-0.21	-0.49
LA03	2.17	2.19	2.25	2.50	2.00	0.09	-0.10	0.66
	Wheat protein, g kg^{-1}							
ST01	126	120	130	152	112	8	0.44	-0.16
ST02	131	130	131	159	116	8	0.78	1.16
AL02	143	150	144	159	132	5	0.09	-0.44
ST03	137	138	141	156	129	6	0.19	-0.73
LA03	138	141	141	152	131	4	-0.07	0.10
	NIR-hardness index ‡							
ST01	29	68	46	105	14	22	0.41	-1.02
ST02	43	56	47	89	29	13	0.74	0.12
AL02	38	75	53	105	32	16	0.56	-0.62
ST03	45	61	53	96	33	16	0.48	-1.17
LA03	45	58	53	92	34	14	0.43	-1.03
	SKCS-hardness index ‡							
ST01	0	56	32	87	0	23	0.17	-1.43
ST02	25	52	42	91	10	22	0.22	-1.44
AL02	13	58	40	91	6	20	0.22	-1.31
ST03	24	59	42	92	11	18	0.28	-1.09
LA03	32	61	43	85	16	17	0.24	-1.21

† Population of 132 F_{12} recombinant inbred lines; SD = standard deviation among RIL means

‡ Extremely soft = 0, extremely hard = 100

Table 3. QTLs detected in more than one environment (italicized) by composite interval mapping analysis for the RIL population, Ning7840 x Clark, evaluated in various Oklahoma environments from 2001 to 2003 (bold = major QTLs, LOD > 3; non-bold = minor QTLs, 2 < LOD ≤ 3). QTLs detected in only one environment are given in plain type.

Trait	Symbol	Environments							Total†	Consistent Linkage group‡
		2001	2002			2003				
		Stillwater	Stillwater	Lahoma	Altus	Stillwater	Lahoma	Altus		
Physical factors										
Test weight	TW	*2BS*, ***5A***, ***5B,*** ***6B***, *7DL*	***5A***, ***5B***, *7DL*	*3BL, 5A,* ***5B***	*2BS, 5A,* ***5B***, 4AL, **4B**	*5A,* 3BL2	*2BS, 3BL,* ***5A***, *5B,* ***5A***, ***5B***, *6B*	*7DL*	**11**-15	***5A, 5B***
Kernel weight	KW	***1B***, ***5A***, *5D*, ***6A***, 7A	***1B***, *2BS*, ***5A***, ***5D***, ***6A***, **3BS**, 7BL		***1B***, *4B*, ***5A***, ***5D***, ***6A***, *6B*	***2BS***, *4B*, ***5A***, ***6A***, 1AL	*2BS*, ***4B***, ***6A***, ***6B***		**18**-9	***5A, 6A***
Kernel diameter	KD	***5A, 6A***, **5D**, 7BS2, **7DL**	*2BS*, ***5A***, ***6A***, 1B, 3BL		***1AL***, ***4B***, ***5A***, ***6A,*** 3BS	***1AL***, *2BS, 4B*, ***5A***, ***6A***	***4B***, ***6A***, 2DL, **6B**		**16**-8	***5A, 6A***
Class factors										
Wheat protein	WP	***7BL***, **2BL**, 5B	*3AS2, 4B, 7BL,* 3BS		*3AS2, 4B, 7BL*	***4B***, ***6B,*** 5D	*3AS2, 4B,* 7A		**4**-12	***4B***
NIR-hardness index	HI	***5B***, *7BS2, 7DL2,* 3BS, 7BL	*2BS*, ***3AS2***, *6A*		*2DL*, ***3AS2***, *6A, 7BS2,*	***2DL***, *7BS,* ***7BS2***	***3AS2***, *5B, 7DL2,* 3BL2, 4B		**6**-14	***3AS2,*** ***7BS2***
SKCS-hardness index	HI-SK	***3AS2***, *4B, 5B,* 3BL	***3AS2***, *6A*, **3BS**		***3AS2***, *4B, 6A*	*3AS2,* 7BL, **7BS2**, **7DL2**	*3AS2*, ***4B,*** *5B,* 3BL2		**7**-11	***3AS2***
Total		**14**-13	**12**-13	**1**-2	**12**-14	**12**-10	**10**-15	**1**-2	**62-**69	

† Incidence of a major QTL identified across all environments (boldface) — incidence of a minor QTL identified across environments.

‡ Linkage group(s) with the highest consistency across environments.

Table 4. Primary genomic regions and their associated additive gene effects for wheat quality factors identified by composite interval mapping with a minimum LOD threshold of 3.0. Peaks of these linkage groups are listed in bold italics in Table 3.

Linkage group	Position	Marker interval	LOD	a†	R^2
	cM				%
Test weight				kg hL^{-1}	
4B	83	*AGG.CAG1/BARC20.4BS-7BL*	4.0	-0.77	9.7
5A	29	*BARC180.5AS-6BL/ACG.GAC1.2*	6.5	1.14	20.6
5B	149	*AGC.GCG12/AAG.CTC4*	6.1	-0.92	15.5
6B	24	*AGC.GCG1/GTG.CTT1*	3.7	0.71	9.0
Kernel weight				mg	
1B	68	*AGG.CTC13/CTCG.AGC9*	8.6	-1.24	19.3
2BS	0	*BARC35.2BS/AGT.CAGT5*	3.1	0.74	7.5
3BS	51	*ACT.CAT3/XGWM493.3BS*	3.5	0.74	7.2
4B	85	*BARC20.4BS-7BL/AAC.GCAG4*	3.4	-0.55	9.4
5A	41	*ACG.GAC6/ACA.CTA4*	5.7	1.26	13.7
5D	61	*AGT.CTG10/AGG.CTC4*	4.1	-0.96	9.8
6A	80	*AAC.CTG5/AAC.CTG5*	9.9	1.42	26.6
6B	91	*ACT.GCG11/ACA.CTG16*	3.6	0.60	9.4
Kernel diameter				mm	
1AL	64	*AAG.CTA11/CTCG.CGAC6*	6.4	-0.05	14.2
4B	81	*AGG.CAG1/BARC20.4BS-7BL*	4.2	-0.03	10.9
5A	45	*ACG.GAC6/ACA.CTA4*	7.3	0.06	16.9
5D	59	*AGT.CTG10/AGG.CTC4*	3.2	-0.05	9.5
6A	69	*CTCG.GTG2/CTCG.GTG2*	9.1	0.07	25.0
6B	91	*ACT.GCG11/ACA.CTG16*	4.5	0.03	11.8
7DL	4	*ACTG.GCG8/ACA.CTA14*	3.2	0.05	7.4
Wheat protein				g kg^{-1}	
2BL	38	*ACT.CAT4/GCTG.ACGC2*	3.5	-2.6	9.4
4B	83	*BARC20.4BS-7BL/AAC.GCAG4*	4.2	-2.5	12.5
6B	101	*ACT.CAT2/AAC.CTG2*	3.7	2.5	10.9
7BL	14	*CTCG.CTG9/BARC63.7BL*	3.1	2.5	8.6
NIR-hardness index				0-100‡	
2DL	59	*BARC159.2DL/ACA.GCG1*	3.4	5.2	10.0
3AS2	83	*XGWM2.3AS/AGC.CTC1*	4.3	-5.0	13.8

Linkage group	Position	Marker interval	LOD	a†	R^2
	cM				%
5B	42	*AGT.CTG2/ACC.AGC7*	3.1	-7.1	10.1
7BS2	32	*AGT.CAGT6/XGWM537.7BS-5BL*	5.4	-7.1	17.6
SKCS-hardness index				0-100‡	
3AS2	83	*XGWM2.3AS/AGC.CTC1*	4.5	-8.0	14.7
3BS	46	*AAG.AGC10/CTCG.CTC9*	3.9	-9.3	10.7
4B	13	*ACT.CTC7/BARC114.4BL*	3.6	-5.8	11.1
7BS2	25	*AGT.CTG3/AGT.CAGT6*	3.1	-6.2	9.6
7DL2	0	*BARC97.7DL/AAC.CGAC9*	3.6	7.0	11.8

† 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.

‡ Extremely soft = 0, extremely hard = 100

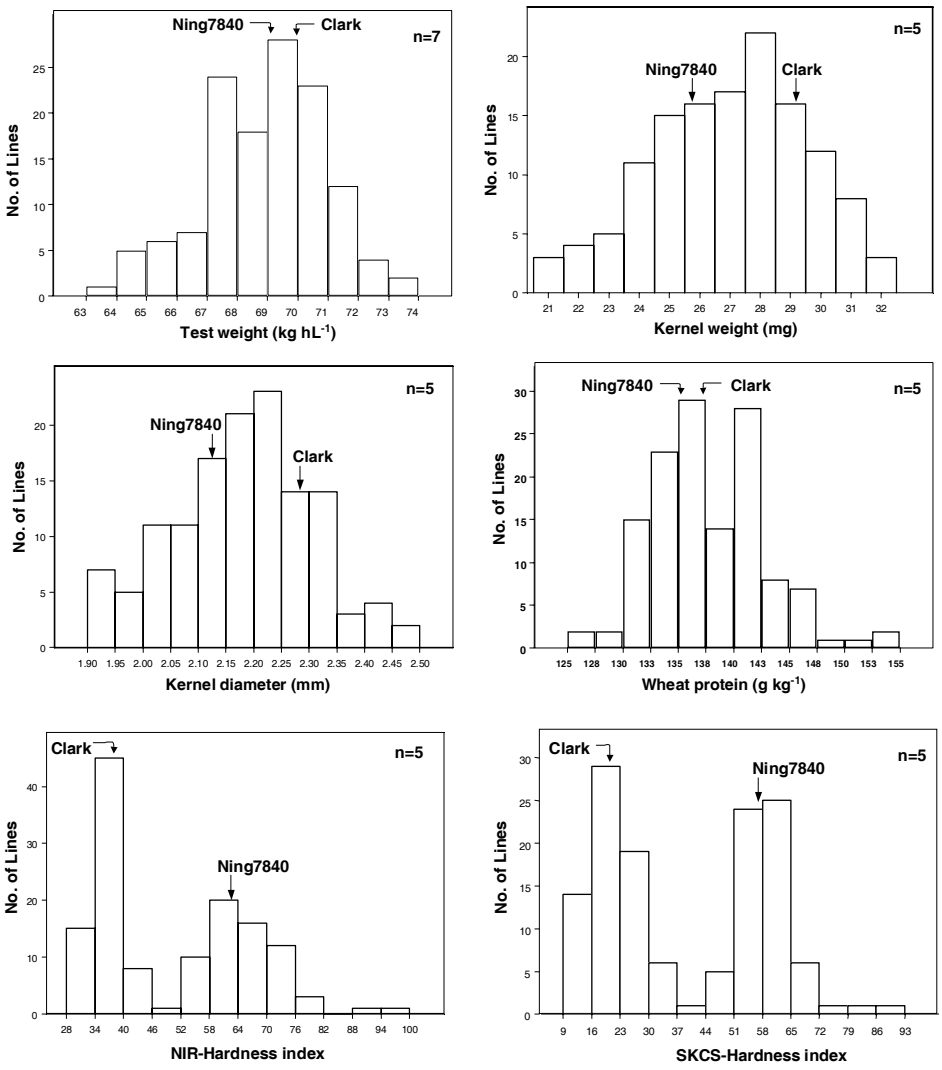

Fig. 1. Frequency distributions for wheat quality traits of 132 RILs averaged across *n* environments. Parental means of Ning7840 and Clark are indicated by arrows.

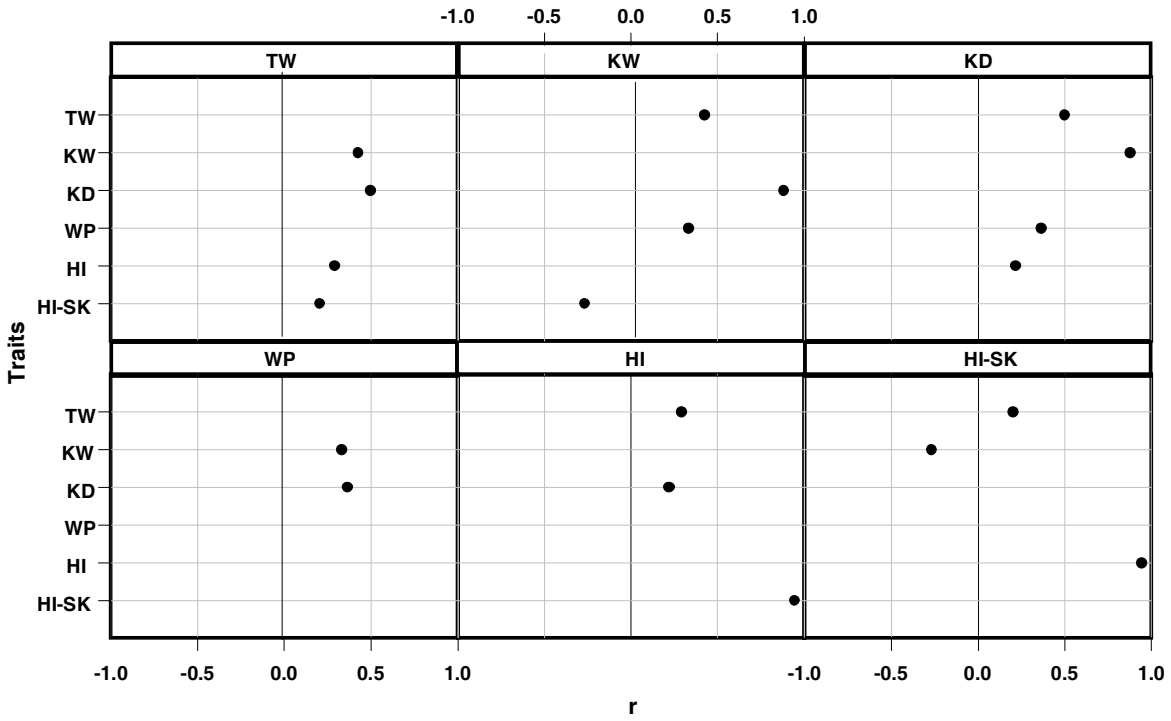

Fig. 2. Summary of phenotypic correlation coefficients for wheat quality traits and class factors for the RIL population, Ning7840 x Clark, evaluated in various Oklahoma environments from 2001 to 2003. Only significant *r*-values ($P < 0.05$) are shown in the plot. Traits are test weight (TW), kernel weight (KW), kernel diameter (KD), wheat protein (WP), NIR-hardness index (HI), and SKCS-hardness index (HI-SK).

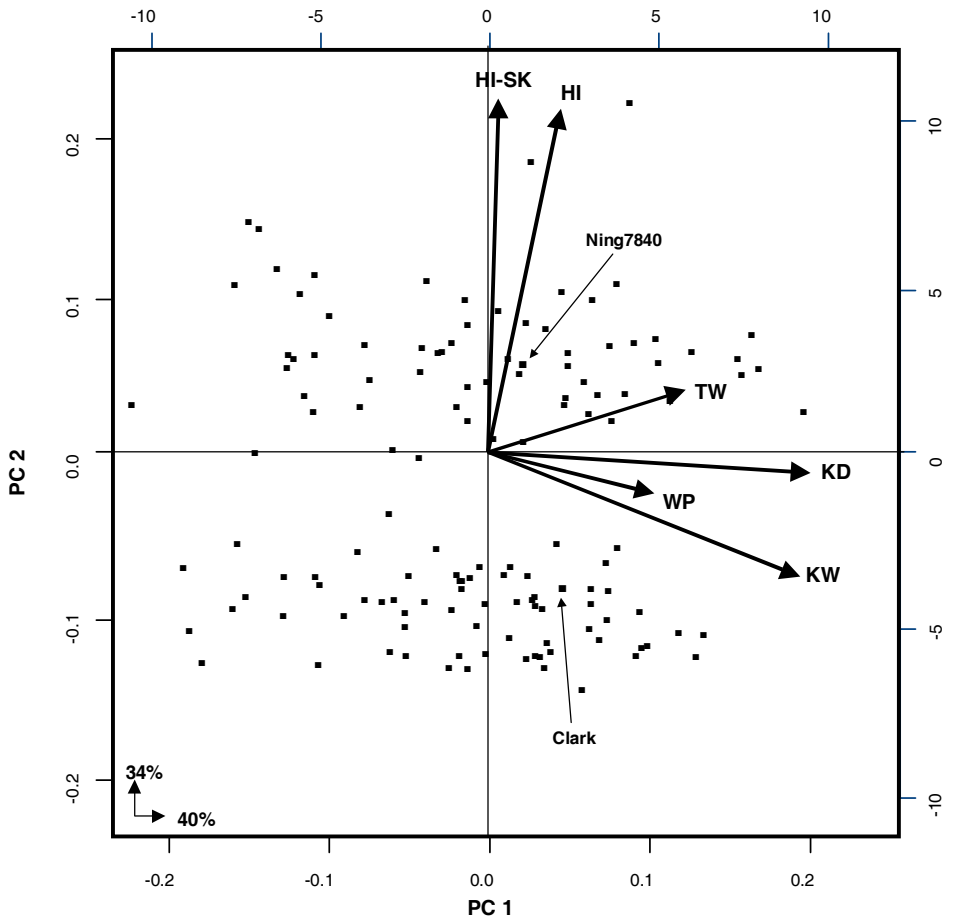

Fig. 3. Principal component (PC) analysis biplot summarizing the relationship among wheat quality traits for the RIL population, Ning7840 x Clark, evaluated in various Oklahoma environments from 2001 to 2003. Traits are test weight (TW), kernel weight (KW), kernel diameter (KD), wheat protein (WP), NIR-hardness index (HI), and SKCS-hardness index (HI-SK).

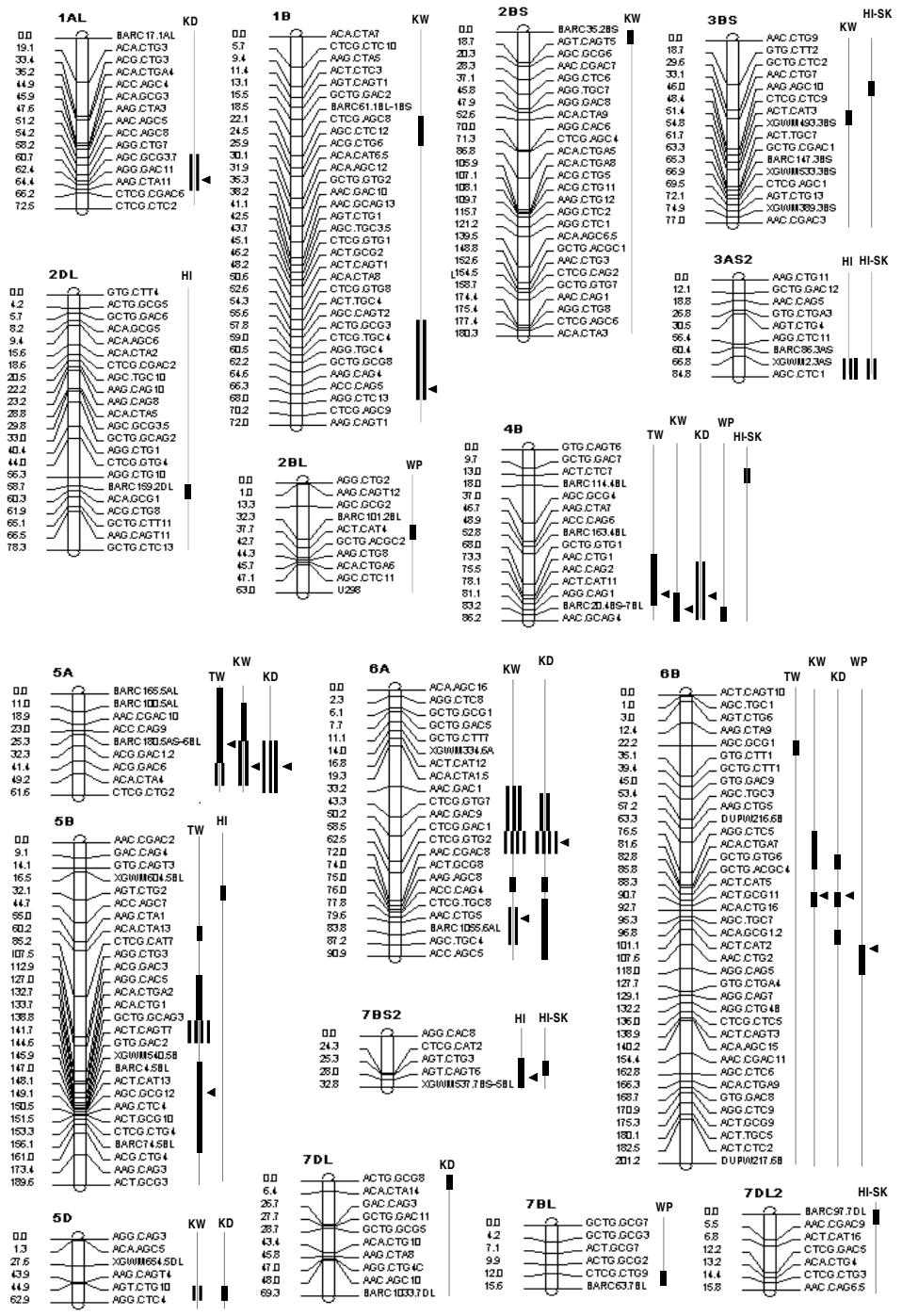

Fig. 4. Primary genomic regions of identified major QTLs (LOD > 3) affecting wheat quality traits and class factors for the Ning7840 x Clark RIL population evaluated in various Oklahoma environments from 2001 to 2003. Bars indicate the number of environments for which the same marker interval was detected. Triangles indicate the interval exhibiting the peak LOD value

CHAPTER III

MODEL EXPANSION WITH EPISTATIC AND QTL x ENVIRONMENT INTERACTION EFFECTS IN MAPPING QTLs FOR WHEAT YIELD AND QUALITY TRAITS

ABSTRACT

Additive effects, epistatic effects, and their environmental interactions of QTLs are crucial genetic components of quantitative traits. In this study, QTLMapper 1.0 was used to analyze the genetic basis of yield and quality related traits in wheat. The objective was to dissect effects of QTLs into additive and epistatic components as well as their interactions of the QTLs with environments, and to evaluate the relative magnitude of these components. A recombinant inbred line population from Ning7840 x Clark tested in replicated field trails in five environments was subjected to QTL analysis based on mixed-model. A total of 20 main effect QTLs and 37 epistatic digenic interactions with relative magnitude (h^2) > 2 were detected for yield, plant height, test weight, kernel weight, protein content, and hardness index. Important QTLs with additive effects only were identified in linkage groups 5A (yield), 5A, 2BS (test weight), and 5B (kernel weight). The h^2 of additive QTLs was larger than h^2 of epistatic QTLs for kernel weight and yield, while for protein content, plant height and especially for hardness index was the opposite. Epistatic QTLs tended to show a greater level of QTL x environment interaction than additive QTLs, suggesting that epistatic QTLs are more prone to environmental influence than additive QTLs. For all the loci with epistatic effect, 46% did not have significant additive effects on their own but were involved in digenic interactions. These loci might play the role of modifying agents that tend to activate or modify the action of other loci. This study attempted to assess the genetic components that may be crucial to consider in marker-assisted selection.

INTRODUCTION

Economically important traits such as yield and quality in wheat (*Triticum aestivum* L.) are genetically complex, are governed by loci that have quantitative effects on the phenotype, and are highly sensitive to genetic background and environmental factors (Barton and Keightley, 2002; Walsh, 2002). Even so, extraordinary rates of genetic improvement have been achieved for intricate quantitative traits in plants despite the limitation of phenotype being an imperfect predictor of breeding value (Dekkers and Hospital, 2002). Advances in molecular-marker techniques, and the availability of high-density linkage maps together, led to the discovery of quantitative trait loci (QTLs) for important traits in wheat (Marshall et al., 2001; Langridge et al., 2001; McIntosh et al., 2003). Presumably due to the lack of appropriate methodology, marker-based QTL analysis for quantitatively inherited traits is typically conducted under the hypothesis of additive main effects only, and assuming the absence of epistasis and QTL x environment interaction. Hence main effects are assumed to be expressed in the same way across different environments.

Epistasis describes any interaction between two or more loci, such that the phenotype of any genotype cannot be predicted simply by summing the genetic effects of individual loci (Carlborg and Haley, 2004). Accumulation of favorable epistatic combinations is considered critical to the evolution of adaptiveness in plants; furthermore, it is considered even more important in self-pollinated crops such as wheat which has evolved from a fixed genome with relatively few recombination events (Goldringer et al., 1997).

Goldringer et al. (1997) and Carlborg and Haley (2004) argue that epistasis should be accounted for in complex trait analysis because genetic models with no epistatic terms

could lead to a biased estimation of QTL parameters. The knowledge of the relative proportion of non-additive variance with respect to additive might be crucial for adopting appropriate breeding strategies.

One of the significant hurdles to extending QTL analysis to breeding application is the manifestation of environmental instability of mapped QTLs. The QTLs detected in one environment but not in another might indicate QTL x environment interaction, but it is impossible to assess the contribution of QTL x environment interaction to phenotype variation by simply comparing QTLs detected in multiple environments. Significant epistasis and QTL x environment interaction effects were reported in many crops (Cao et al., 2001; Kamoshita et al., 2002; Campbell, 2004). Xing et al. (2002) and Li et al. (2003) point out that interactions among loci and environmental factors make a substantial contribution to variation in complex traits and therefore should be accounted for in complex trait studies.

Continuing advances in methodologies for analysis offer direct mapping of QTLs with additive and epistatic effects, as well as their QTL x environment interaction, based on mixed linear model approaches (Wang et al., 1999). The software QTLMapper version 1.0 was developed (Wang et al., 1999; Zhu, 1999) for that purpose. In this study phenotypic and molecular marker data of 132 RILs were subjected to two-locus analysis via QTLMapper. Previous studies revealed highly significant QTLs affecting agronomic and milling quality traits in this population, but several traits appeared to be influenced by multiple QTLs with minor and/or major effects that were not highly consistent across environments (Marza et al., 2005a, 2005b). Hence, the objectives of the study were to further characterize yield and quality traits for the presence of epistatic QTLs and QTL x environment interactions, and to evaluate the relative magnitude of these components.

MATERIALS AND METHODS

Genetic material and field experiments

The population used in this study consisted of 132 F_{12} recombinant inbred lines (RILs) derived by single-seed descent from a cross between Ning7840 and Clark (Bai et al. 1999). The details of the Ning7840 x Clark population are described in previous report (Marza et al., 2005a). Briefly, Ning7840 is hard facultative wheat from China. Clark is a soft red winter wheat cultivar developed at Purdue University (Ohm et al., 1988). The RIL population and the two parental genotypes were grown at three Oklahoma locations (Stillwater, Lahoma, and Altus), using a replicates-in-sets design with three replications and with a plot size of 1.4 m^2 planted at a density of 58 kg ha^{-1}.

The study utilized data for six traits measured in five Oklahoma environments: Stillwater in 2001, 2002, and 2003 (ST01, ST02, ST03); Altus, 2002 (AL02); and Lahoma, 2003 (LA03). Grain yield (GY) was measured as the weight of wheat grain harvested from the entire plot area. Plant height (HT) was measured at harvest maturity from ground level to the tip of the spike, excluding awns. Test weight (TW) was measured in kg hl^{-1} as the weight from a 0.95-L container. The single kernel characterization system 4100 (SKCS) (Perten Instruments North America, Inc., Springfield, IL) was used to estimate kernel weight (KW, mg) and hardness index (HI, on a scale of 0 = extremely soft to 100 = extremely hard), using a sample of 300 kernels from each plot. Grain protein content (WP, g kg^{-1}) was determined by near-infrared reflectance (NIR) spectroscopy according to AACC method 39-70a (AACC, 1995). The test was performed using ground, whole wheat, 9-g samples from each replicate.

Molecular markers and QTL analysis

Two kinds of DNA markers representing 410 loci were used to develop the genetic linkage map using the Mapmaker program (Macintosh V2.0, Lander et al., 1987). The SSR marker assay followed the method described by Bai et al. (2004) and the AFLP assay was conducted as described by Bai et al. (1999). The genetic linkage map that was used for QTL mapping of this population contained 363 AFLP and 47 SSR markers distributed among 29 linkage groups covering 2,223 cM with an average distance of 5.4 cM between markers (Marza et al., 2005a).

The two-locus analysis that tests the additive main effect and additive x additive epistatic effects, as well as their environmental interaction effects, was performed using QTLMapper version 1.0 (Wang et al., 1999). The phenotypic value of the k = 132 RILs in l = 5 environments can be partitioned by the following mixed linear model (Zhu, 1999):

$$y_{kl} = \mu + a_i x_{A_{ik}} + a_j x_{A_{jk}} + aa_{ij} x_{AA_{ijk}} + u_{E_{kl}} e_{E_l} + u_{A_i E_{kl}} e_{A_i E_l} + u_{A_j E_{kl}} e_{A_j E_l} + u_{AA_{ij} E_{kl}} e_{AA_{ij} E_l}$$

$$+ \sum_{f(l)} u_{M_{fk(l)}} e_{M_{f(l)}} + \sum_{n(l)} u_{MM_{nk(l)}} e_{MM_{n(l)}} + \xi_{kl},$$

In which μ is the population mean; a_i and a_j are the additive fixed effects of two putative loci Q_i and Q_j, respectively; aa_{ij} is the additive x additive epistatic fixed effect between the loci; $x_{A_{ik}}$, $x_{A_{jk}}$ and $x_{AA_{ijk}}$ are the coefficients for these genetic main effects; e_{E_l} is the random effect of environment l with a coefficient $u_{E_{kl}}$; $e_{A_i E_l}$ (or $e_{A_j E_l}$) is the random additive x environment interaction effect with coefficient $u_{A_i E_{kl}}$ (or $u_{A_j E_{kl}}$) for Q_i (or Q_j); $e_{AA_{ij} E_l}$ is the random epistatic x environment interaction effect with a coefficient $u_{AA_{ij} E_{kl}}$;

$e_{M_{f(l)}}$ is the random effect of marker *f* nested within the *l*-th environment with a coefficient $u_{M_{fk(l)}}$, $e_{MM_{n(l)}}$ is the random effect of the *n*-th bi-marker interaction nested within the *l*-th environment with a coefficient $u_{MM_{kn(l)}}$; and ξ_{kl} is the random residual effect. The marker factors $e_{M_{f(l)}}$ and $e_{MM_{n(l)}}$ in the model are used to absorb the additive and epistatic effects of background QTLs.

The QTL analysis by means of QTLMapper v 1.0 was carried out in three steps. First, significant (*P*=0.005) markers were identified across the genome using stepwise regression based on single-marker genotypes for putative main-effect QTL and on all possible marker pairs for epistatic QTL in an individual environment. Second, all putative main-effect and epistatic QTL were identified in putative QTL regions. The associated QTL effects and test statistics were simultaneously estimated at the positions of respective LOD peaks in individual putative QTL regions using the restricted maximum likelihood (LR) method (LOD = 0.217 LR) (Wang et al., 1999). Additive and epistatic main QTLs were filtrated under the threshold *P* = 0.005. Third, genetic effects were further tested by a t-test with the jackknifing re-sampling procedure. QTLs were reported when genetic main effects (*a* and *aa*) or QTL x environment (QE) interaction effects (*ae* and *aae*) were significant (*P* = 0.005). The proportion of phenotypic variance caused by a specific genetic source (*a*, *aa*, *ae,* and *aae*) was calculated and interpreted as an estimate of narrow sense heritability (h^2) contributed by that source.

RESULTS AND DISCUSSION

Phenotypic summary

Between the parents, Clark produced higher yield (2595 kg ha^{-1}), heavier kernels (29.7 mg), and marginally taller plants (80 cm) across environments (P<0.05) compared to Ning7840 (2219 kg ha^{-1}, 26.3 mg, and 76 cm). As expected for soft red winter (SRW) wheat, Clark had lower kernel hardness index (18) than Ning7840 (57). These parents produced similar values for test weight (71 vs. 70 kg hL^{-1} for Clark and Ning7840 respectively) and protein content (136 g kg^{-1}). Most traits described here segregated continuously, and both skewness and kurtosis values were less than 1.0. The only exception was hardness index, which exhibited bimodal distribution. Transgressive segregation occurred in both directions for all traits, indicating gene dispersion between the two parents.

QTL mapping

The genomic proportion of the 29 linkage groups used here were 9 (702.0 cM), 13 (1222.2 cM), and 7 (298.3 cM) for A, B, and D respectively. In this study we detected a total of 90 and 177 putative QTLs with additive and epistatic effects respectively. For all traits the total number of QTLs with main effect were 28 (31%), 53 (59%) and 9 (10%) for genomes A, B, and D respectively. Most of the main effect QTLs were associated with genome B and least with D. The genome distribution of the epistatic QTLs was not different from that of additive effects 56 (32%), 100 (56%), and 21 (12%) for genomes A, B, and D respectively. The highest number of additive QTLs was concentrated in homologous chromosomes 3 and 7, whereas for epistatic QTLs linkage groups associated with homologous chromosomes 3 and 6 were the most common.

QTLs with additive main and additive x environment interaction effects

The two QTL interaction analysis resolved a total of 14 to 17 significant ($P <$ 0.005) QTLs with additive main effect among the six traits (Figs. 1a and 2). For grain yield and kernel weight, nine additive x environment interaction effects were detected. This was the highest frequency of *ae* interactions for any trait as might be expected given their typically low heritability. Only two interactions were detected for kernel hardness. Collectively, the additive effects explained 13 to 56% of the phenotypic variation, while the additive x environment effects accounted for 1 to 15% of the phenotypic variation (Fig. 1b).

Wang et al. (1999), in testing the power of the mixed model approach for the two-locus QTL analysis, indicated that QTLs with large additive and/or epistatic effects with relative magnitude $h^2 > 6\%$ can almost always be detected and their positions and effects accurately estimated. On the other hand QTLs with $h^2 < 2\%$ are considered largely unstable. In our study, we first quantified the total number of significant additive and/or epistatic effects ($P < 0.005$, equivalent to LOD = 2.79), including their environmental interactions (Fig. 1a) and their total relative magnitudes (Fig. 1b). Similarly, the full range of intervals of additive and /or epistatic effects depicted in Figure 2 also include all significant ($P < 0.005$) effects. However, further discussion will focus on the more consistent additive and/or epistatic effects as recommended by Wang et al. (1999). We summarized in Tables 1 and 2 only those QTLs which explained > 2% of the phenotypic variation.

For grain yield, two additive main effects were identified in linkage groups 4AL (*AGG.CTG11/GCTG.GTG5*) and 5A, accounting for 6 and 2% of the phenotypic variation respectively; for both QTL the allele from Clark increased the phenotypic value

(Table 1). The role of chromosome 4AL (Araki et al., 1999), and that of chromosome 5A (Kato et al., 2000; Marza et al., 2005a) for yield have been particularly emphasized. The effect of the QTL in linkage group 5A was exclusively additive. These QTLs identified for yield were slightly sensitive to environmental variation. The additive x environment interaction effect for 4AL with environment AL02 was negative, while the interaction effect of the QTL in 5A with environment LA03 was positive; yet their relative magnitudes were low ($h^2 < 1\%$; Table 1).

Three additive main effects for plant height explaining 7, 4, and 3% of the phenotypic variation were mapped in linkage groups 6A (*AGC.TGC4/ACC.AGC5*), 4B, and 1B respectively (Table 1). These regions have been widely reported for this trait (Borner et al., 2002; Huang et al., 2003, 2004; Marza et al., 2005a). The QTL in linkage group 6A exhibited the strongest *ae* interaction, involving four of the five environments and explaining 4% of the phenotypic variation; in contrast 4B and 1B were insensitive to environmental variation. For grain yield and plant height, all major QTLs detected by single-locus analysis for the same population (Marza et al., 2005a) was confirmed here. However, an additional QTL for plant height in linkage group 1B was discovered here ($h^2 = 3\%$), which went undetected in the single-locus analysis.

For test weight, the two most important QTLs with additive effects in linkage groups 5B and 5A explained 4 to 6% of the phenotypic variation (Table 1). These were found associated with kernel packing efficiency (5B) and kernel density (5A) in similar regions in the earlier report (Marza et al., 2005b). The Clark allele increased test weight in 5A, whereas the Ning7840 alleles increased test weight in 5B and two other important QTLs in linkages groups 2BS and 4B. The QTLs on 2BS and 5A were reported in similar regions by Campbell et al. (1999). Interestingly, QTLs in linkage groups 5A and

2BS were exclusively associated with additive effects. For test weight, three of the four QTLs interacted with environments, each accounting for 1% of the phenotypic variation, suggesting that test weight was relatively insensitive to environmental variation.

In this study, extraordinarily large additive effects contributed to variation in kernel weight (Fig. 1b), suggesting that more than half of the variation for this trait was fixable and that the associated QTLs should be particularly useful in marker-aided breeding. The three most important QTLs for kernel weight were mapped in linkage groups 6B (*ACT.GCG11/ACA.CTG16*), 6A (*CTCG.GTG2/AAC.CGAC8*), and 1B (*CTCG.AGC9/AAG.CAGT1*), explaining 16, 11, and 7% of the phenotypic variation, respectively, and with the alleles from Clark increasing the phenotypic value (Table 1). The identified intervals for 6B and 6A (Fig. 2) were found in the exact same genomic positions based on single-locus analysis (Marza et al., 2005b), which corroborates the argument of Wang et al. (1999) that QTLs with $h^2 > 6\%$ will always be detected in the same position. Additional QTLs with $h^2 > 2\%$ were identified in linkage groups 5D, 5A, 5B, 3BL, and 7A. The effects of QTLs on 5B and 7A were exclusively additive. Among the traits considered here, kernel weight was the trait with the largest additive x environment effect (Fig. 1b). Most of the QTLs with additive effect for kernel weight exhibited QTL x environment interaction; but two exceptional QTLs in linkage groups 5B and 7A were insensitive to environmental variation; moreover, they associated with additive effects only.

The lack of phenotypic differences between parental lines for wheat protein was reflected in the relatively low magnitude of effects associated with the identified QTLs (Table 1 and Fig. 1b). Additive effects of two important QTLs (linkage groups 4B and 3AS2) accounted for only 2% of the phenotypic variation, and each QTL exhibited

positive additive x environment interaction with LA03 and AL02, respectively. The results of two-locus QTL analysis for hardness index identified one QTL on linkage group 3AS2 (*XGWM2.3AS/AGC.CTC1*) with a relatively large additive effect. The Ning7840 allele increased the phenotypic value (Table 1) as expected given Clark's SRW classification. A QTL for this trait was reported in the same chromosomal arm location by Campbell et al. (1999). The identified QTL was insensitive to environmental variation and did not map to the chromosomal region of 5DS believed to explain major differences in hardness of soft versus hard wheat (Morris, 2002).

In general, most of the QTLs with additive effects identified previously based on single-locus analysis (Marza et al., 2005b) were found in the same vicinity of the QTLs identified here (Fig. 2). More striking was the overall lack of sensitivity to environmental variation of those QTLs associated with additive effects only, which may be one of the virtues of two-locus analysis that may help uncover QTLs amenable for marker-assisted selection. Additive x environment interaction presumably arose from differential gene expression in different environments, or from QTL expression in one environment but not in another. The pattern of differential expression of additive x environment interaction with no direction of the effects in this study appears to be very complex.

QTLs with epistatic and epistatic x environment interaction effects

Among all traits, the two-locus QTL analysis resolved a total of 6 to 24 QTLs with significant ($P < 0.005$) additive x additive epistatic (*aa*) effects and 2 to 10 QTLs with additive x additive x environment (*aae*) interaction effects (Fig. 1a and Table 2). In either case, grain yield accounted for relatively few *aa* and *aae* effects, whereas epistasis was prominent for hardness index. Virtually all of the phenotypic variation for hardness was epistatic. For the other traits, epistatic effects accounted for 10 to 40% of the total

phenotypic variation. Epistatic x environment interaction effects accounted for < 16% of the phenotypic variation, and for grain yield this component comprised only 1% (Fig. 1b).

The digenic epistatic interaction of two loci in linkage group 4AL for grain yield accounted for 5% of the phenotypic variation with little sensitivity to environmental variation (Table 2, Fig. 2). For plant height, five digenic epistatic interactions explained 2 to 5% of the phenotypic variation. They included five additive main effects (6B, 6A, 3BL, 4B, and 3AS2) but four others produced non-significant additive effects. The latter have been referred to as 'modifier factors', meaning that gene expression of some QTLs could be induced by the environment (Cao et al., 2001). In addition, important epistasis x environment interactions between two intervals in linkage group 6A explained 12 % of the phenotypic variation; interactions with environments ST01, ST02, and ST03 were negative, whereas the interaction with LA03 was positive.

For test weight, four digenic epistatic QTLs were identified in linkage groups 3BL, 5A, 5B, 6B, 7A, and 7DL and accounted for 2 to 5% of the variation. None of these influential epistatic effects interacted with environments. For kernel weight, eight epistatic QTLs (including 8 of the additive QTLs) were distributed in six linkage groups (6A, 6B, 5B, 4B, 3BL2, and 7BS) and were involved in five digenic interactions explaining 2 to 12% of the variation (Table 2 and Fig. 2). A single digenic interaction between QTLs 5B and 7BS was positive, while all the remaining QTLs produced negative interaction effects. The epistatic x environment interaction for this trait appeared to be induced by the effects of years more than sites. Negative effects were associated with ST02 and AL02, while positive effects were associated with ST03 and

LA03. The largest effect (5%) was produced by the digenic interaction of two loci in linkage group 6A.

For protein content 14 QTLs were identified in seven digenic combinations distributed across 12 linkage groups (Table 2 and Fig. 2). Seven of these 12 loci coincided with QTLs showing additive main effects (Table 1). Among the seven digenic combinations, four showed epistatic x environment effects with one to three of the environments explaining < 3% of the phenotypic variation. Epistatic effects were positive at four pairs of loci indicating that recombination of the parental alleles increased protein content. Altogether, 24 digenic epistatic interactions were detected for hardness index, explaining 4 to 11% of the variation (Table 2, Fig. 2). Among them, 11 pairs had at least one additive effect at one site, but five pairs showed no significant additive effects. Only six pairs showed epistatic x environment interaction effects in one to two environments. Gene interactions obviously play a major role in hardness expression for this population. To our knowledge, characterization of this trait for epistatic effects was not addressed. Interactions between QTLs and other modifying loci might be the prevalent form of epistasis (Yu et al., 1997).

Overall, the model containing *a*, *aa*, *ae*, and *aae* effects constituted varying proportions of phenotypic variation, depending on the trait. For grain yield, the proportion was lowest (28%). For plant height, test weight, and protein content, the model was much more effective (53 to 77%), whereas for kernel weight and hardness index, a digenic model with additive effects was sufficient (122 to 148%). As would be expected, we were more successful in dissecting a component of grain yield and kernel weight, than grain yield itself. In comparing genetic effects with non-genetic effects, and averaging across traits, the combined *a* and *aa* effects outweighed the *ae* and *aae* effects

by four to one (80% vs. 20%). Still, 23 to 72% of the phenotypic variation for yield, plant height, test weight, and protein content remains unexplained and may be attributed either to higher order interactions or environmental variation. It is also possible that some of the QTLs for these traits escaped detection because the alleles for these QTLs did not differ in the Ning7840 and Clark parents. Additional factors for the high percentage of unaccounted variance may be due to the genome coverage (poor for some linkage groups).

Knowledge of the proportions of additive vs. epistatic effects is clearly very important for the purpose of breeding and marker-assisted selection. The importance of epistasis in determining quantitative trait variation has been well demonstrated here by the large number of epistatic QTLs identified and by the involvement of many additive effects in epistasis. Our finding that epistatic QTLs tended to show a greater level of QTL x environment interaction than the additive main effect QTLs is perplexing. It suggests that epistatic QTLs could more likely be influenced by the environment than additive QTLs. Since epistatic effects might be spuriously induced by the environment, selection of these QTLs may not contribute to genetic gains. Hence marker-assisted selection should concentrate more heavily on QTLs with additive main effects.

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Table 1. Summary of estimated additive (*a*) and additive x environment interaction (*ae*) effects of QTLs ($h^2 > 2\%$) for grain yield, plant height, test weight, kernel weight, wheat protein, and hardness index detected by two-locus analysis using QTLMapper for the Ning7840 x Clark RIL population evaluated in Stillwater (ST), Lahoma (LA), and Altus (AL), Oklahoma from 2001 to 2003 (bold = QTLs with additive effects only).

LG-In†	Marker interval	LOD	*a*‡	h^2a §	*ae* ¶ ST01	*ae* ST02	*ae* AL02	*ae* ST03	*ae* LA03	h^2ae §
				%						%
Grain yield, kg ha^{-1}										
4AL_11	*AGG.CTG11/GCTG.GTG5*	9.1	296	5.7			-77			0.2
5A_6	*ACG.GAC1.2/ACG.GAC6*	30.0	176	2.0					139	0.5
Plant height, cm										
6A_21	*AGC.TGC4/ACC.AGC5*	16.0	2.7	7.0	-2.1	-1.0		2.0	1.1	3.6
4B_13	*AGG.CAG1/BARC20.4BS-7BL*	28.1	-2.0	4.0						
1B_8	*CTCG.AGC8/AGC.CTC12*	12.7	-1.6	2.6						
Test weight, kg hL^{-1}										
5B_27	*AAG.CAG3/ACT.GCG3*	23.9	-0.68	5.9		-0.61				1.2
5A_6	*ACG.GAC1.2/ACG.GAC6*	34.3	0.54	3.7			-0.32		0.36	0.8
2BS_19	*GCTG.ACGC1/AAC.CTG3*	10.7	-0.43	2.3						
4B_13	*AGG.CAG1/BARC20.4BS-7BL*	20.5	-0.38	1.9	0.43					0.9
Kernel weight, mg										
6B_17	*ACT.GCG11/ACA.CTG16*	16.4	1.45	16.1	-0.40	-0.41		0.43	0.35	1.2
6A_13	*CTCG.GTG2/AAC.CGAC8*	33.8	1.20	11.1			0.52			0.9
1B_32	*CTCG.AGC9/AAG.CAGT1*	28.4	-0.94	6.9			-0.36		0.92	2.2
5D_5	*AGT.CTG10/AGG.CTC4*	34.3	-0.77	4.6	-0.53	0.35			0.59	1.7
5A_7	*ACG.GAC6/ACA.CTA4*	58.4	0.69	3.7	0.71				-0.61	2.0
5B_14	*ACA.CTG1/GCTG.GCAG3*	4.7	-0.58	2.6						
3BL_3	*AAC.AGC7/AGG.CAC1*	10.4	0.56	2.5		0.43				0.6
7A_5	*CTCG.CAT1/AAG.AGC12*	8.8	0.53	2.2						
Wheat protein, g kg^{-1}										
4B_11	*AAC.CAG2/ACT.CAT11*	18.0	-0.15	2.2					0.12	2.2
3AS2_2	*GCTG.GAC12/AAC.CAG5*	16.1	-0.14	2.0			0.12			0.4

LG-In †	Marker interval	LOD	*a* ‡	h^2a §	*ae* ¶ ST01	*ae* ST02	*ae* AL02	*ae* ST03	*ae* LA03	h^2ae §
				%						%
Hardness index, (0-100)										
3AS2_8	*XGWM2.3AS/AGC.CTC1*	54.5	-7.0	11.3						

† LG-In represent the linkage group and serial number of the initial interval on the corresponding linkage group.

‡ *a* is the additive main effect. A positive value implies Clark allele increasing the corresponding phenotypic value; a negative value implies the Clark allele decreased it.

¶ *ae* is the additive x environment interaction effect.

§ h^2a is the percentage of the phenotypic variation explained by *a*, and h^2ae is the percentage of the phenotypic variation explained by *ae*.

Table 2. Summary of significant ($P < 0.005$ and $h^2aa > 2\%$) epistatic (*aa*) and epistasis x environment interaction (*aae*) effects of QTLs detected by two-locus analysis using QTLMapper for the Ning7840 x Clark RIL population evaluated in Stillwater (ST), Lahoma (LA), and Altus (AL), Oklahoma from 2001 to 2003. (bold = QTLs with significant additive effect).

LG-In$_i$†	Marker interval	LG-In$_j$	Marker interval	LOD	aa_{ij}‡	h^2aa_{ij}§	aae_{ij}¶ ST01	aae_{ij} ST02	aae_{ij} AL02	aae_{ij} ST03	aae_{ij} LA03	h^2 aae_{ij}§
						%						%
Grain yield, kg ha^{-1}												
4AL_11	*AGG.CTG11/GCTG.GTG5*	***4AL_15***	*ACT.CAGT6/AAC.GAC3*	9.1	-277	5.0						
Plant height, cm												
6B_34	*AGG.CTC9/ACT.GCG9*	7BL2_1	*XGWM344.7BL/ACG.CTG7*	34.1	-2.3	5.2		-0.8				0.3
6A_17	*ACC.CAG4/CTCG.TGC8*	***6A_21***	*AGC.TGC4/ACC.AGC5*	16.0	1.6	2.7	-2.1	-1.3		-1.8	5.1	11.7
2DL_21	*AAG.CAGT11/GCTG.CTC13*	***4B_9***	*GCTG.GTG1/AAC.CTG1*	23.1	1.6	2.6						
3BL_1	*CTCG.CTC6/CTCG.CAG5*	7BS_13	*AGC.CAGT4/XGWM68.7BS-5B*	17.7	-1.5	2.4						
2DL2_1	*ACA.CTG5/ACT.CAT14*	***3AS2_5***	*AGT.CTG4/AGG.CTC11*	11.3	-1.5	2.3	1.4				-1.8	1.8
Test weight, kg hL^{-1}												
5B_17	*GTG.GAC2/XGWM540.5B*	***5B_24***	*CTCG.CTG4/BARC74.5BL*	9.4	0.64	5.2						
6B_10	*AAG.CTG5/DUPW216.6B*	7A_8	*GCTG.CGAC2/GCTG.GCG2*	16.6	-0.45	2.5						
3BL_5	*AGC.GCG5/BARC164.3BL*	7DL_7	*AAG.CTA8/AGG.CTG4C*	15.8	-0.40	2.0						
5A_1	*BARC165.5AL/BARC100.5AL*	6B_24	*GTG.CTGA4/AGG.CAG7*	19.3	0.38	2.0						
Kernel weight, g												
6A_17	*ACC.CAG4/CTCG.TGC8*	***6A_21***	AGC.TGC4/ACC.AGC5	41.8	-1.25	12.0		-0.37	-1.22	0.74	0.58	4.9

LG-In$_i$†	Marker interval	LG-In$_j$	Marker interval	LOD	aa_{ij}‡	h^2aa_{ij}§	aae_{ij}¶ ST01	aae_{ij} ST02	aae_{ij} AL02	aae_{ij} ST03	aae_{ij} LA03	h^2 aae_{ij} §
						%						%
6B_15	*GCTG.ACGC4/ACT.CAT5*	***6B_18***	ACA.CTG16/AGC.TGC7	7.8	-0.81	5.0			-0.60	0.54		1.3
5B_25	*BARC74.5BL/ACG.CTG4*	***7BS_8***	E13/AGC.GCG13	5.0	0.63	3.0	0.88		-0.36			1.9
4B_6	*AAG.CTA7/ACC.CAG6*	7BL2_5	ACTG.CTC6/GCTG.CTC11	18.8	-0.59	2.7						
3BL2_11	*BARC77.3BL/AAG.CTA6*	5B_18	XGWM540.5B/BARC4.5BL	9.0	-0.53	2.1						
Wheat protein, g kg^{-1}												
1B_6	*GCTG.GAC2/BARC61.1BL-1BS*	4AL_2	*GCTG.CTT9/BARC170.4AL*	14.2	0.22	4.9						
5B_6	*ACC.AGC7/AAG.CTA1*	7BS_13	*AGC.CAGT4/XGWM68.7BS-5B*	15.3	0.17	2.9	0.26		-0.13		-0.17	3.1
3BL2_6	*CTCG.CAG6/AGC.GCG3*	***5B_14***	*ACA.CTG1/GCTG.GCAG3*	21.3	-0.16	2.5		-0.17				1.5
6A_13	*CTCG.GTG2/AAC.CGAC8*	6A_19	*AAC.CTG5/BARC1055.6AL*	7.6	0.15	2.4						
2BS_5	*AGG.CTC6/AGG.TGC7*	***3BL_13***	*AGG.CTC7/CTCG.CTC11*	23.0	0.15	2.3		0.10		0.13		1.2
3AS2_4	*GTG.CTGA3/AGT.CTG4*	***7A_13***	*ACT.CTC5/ACT.CAT6*	19.1	-0.15	2.2						
2DL_7	*CTCG.CGAC2/AGC.TGC10*	***5D_2***	*ACA.AGC5/XGWM654.5DL*	8.3	-0.14	2.0			0.16			1.7
Hardness index, (0-100)												
6B_11	*DUPW216.6B/AGG.CTC5*	6B_37	*ACT.CTC2/DUPW217.6B*	60.1	7.0	11.4				-3.2		1.0
2BL_6	*GCTG.ACGC2/AAG.CTG8*	6B_4	*AAG.CTA9/AGC.GCG1*	44.0	-6.6	10.0	3.4				-2.8	1.3
5A_1	*BARC165.5AL/BARC100.5AL*	5B_22	*AAG.CTC4/ACT.GCG10*	55.4	-6.0	8.2		-2.2		4.1		1.7
2BL_2	*AAG.CAGT12/AGC.GCG2*	***3AS2_5***	*AGT.CTG4/AGG.CTC11*	31.6	5.9	7.9						
2BS_5	*AGG.CTC6/AGG.TGC7*	***3BL2_7***	*AGC.GCG3/GCTG.CTC1*	33.6	5.6	7.3						
1B_11	*ACA.CAT6.5/ACA.AGC12*	5B_14	*ACA.CTG1/GCTG.GCAG3*	33.7	-5.1	5.9			-2.6	3.7		1.4
1B_10	*ACG.CTG6/ACA.CAT6.5*	***4AL_11***	*AGG.CTG11/GCTG.GTG5*	35.8	4.9	5.6						
5B_2	*GAC.CAG4/GTG.CAGT3*	***6B_17***	*ACT.GCG11/ACA.CTG16*	33.4	-4.7	5.1						
5B_16	*ACT.CAGT7/GTG.GAC2*	6A_21	*AGC.TGC4/ACC.AGC5*	27.1	4.7	5.0						

LG-In$_i$†	Marker interval	LG-In$_j$	Marker interval	LOD	aa_{ij}‡	h^2aa_{ij}§	aae_{ij}¶ ST01	aae_{ij} ST02	aae_{ij} AL02	aae_{ij} ST03	aae_{ij} LA03	h^2 aae_{ij} §
						%						%
6B_32	*ACA.CTGA9/GTG.GAC8*	***7A_5***	*CTCG.CAT1/AAG.AGC12*	25.4	-4.5	4.6	-3.0				2.7	1.3
3A5A_6	*GTG.CTGA2/BARC197.3A-5AL*	***4B_13***	*AGG.CAG1/BARC20.4BS-7BL*	30.1	-4.5	4.5			-2.2	2.5		0.9
1B_1	*ACA.CTA7/CTCG.CTC10*	***7BS2_2***	*CTCG.CAT2/AGT.CTG3*	22.4	-4.2	4.1						
5A_2	*BARC100.5AL/AAC.CGAC10*	***6A_10***	*CTCG.GTG7/AAC.GAC9*	26.5	4.2	4.1						
6A_14	*AAC.CGAC8/ACT.GCG8*	***7BL_2***	*GCTG.GCG3/ACT.GCG7*	27.7	4.1	3.8						
2DL2_2	*ACT.CAT14/AGT.CTG7*	***7DL2_1***	*BARC97.7DL/AAC.CGAC9*	25.8	4.1	3.8						

† LG-In$_i$ and LG-In$_j$ represent the linkage group and serial number of the point tested on the corresponding linkage group.

‡ aa_{ij} is the epistatic effect between points i and j; a positive value indicates that the two-locus parental genotypes had a positive effect (increased phenotypic value), while the recombinants had negative effects.

¶ aae_{ij} is the epistatic interaction effect between points i and j and the environment.

§ h^2aa_{ij} and h^2aae_{ij} are the percentages of the phenotypic variation explained by $aa_{ij,}$ and aae_{ij} respectively

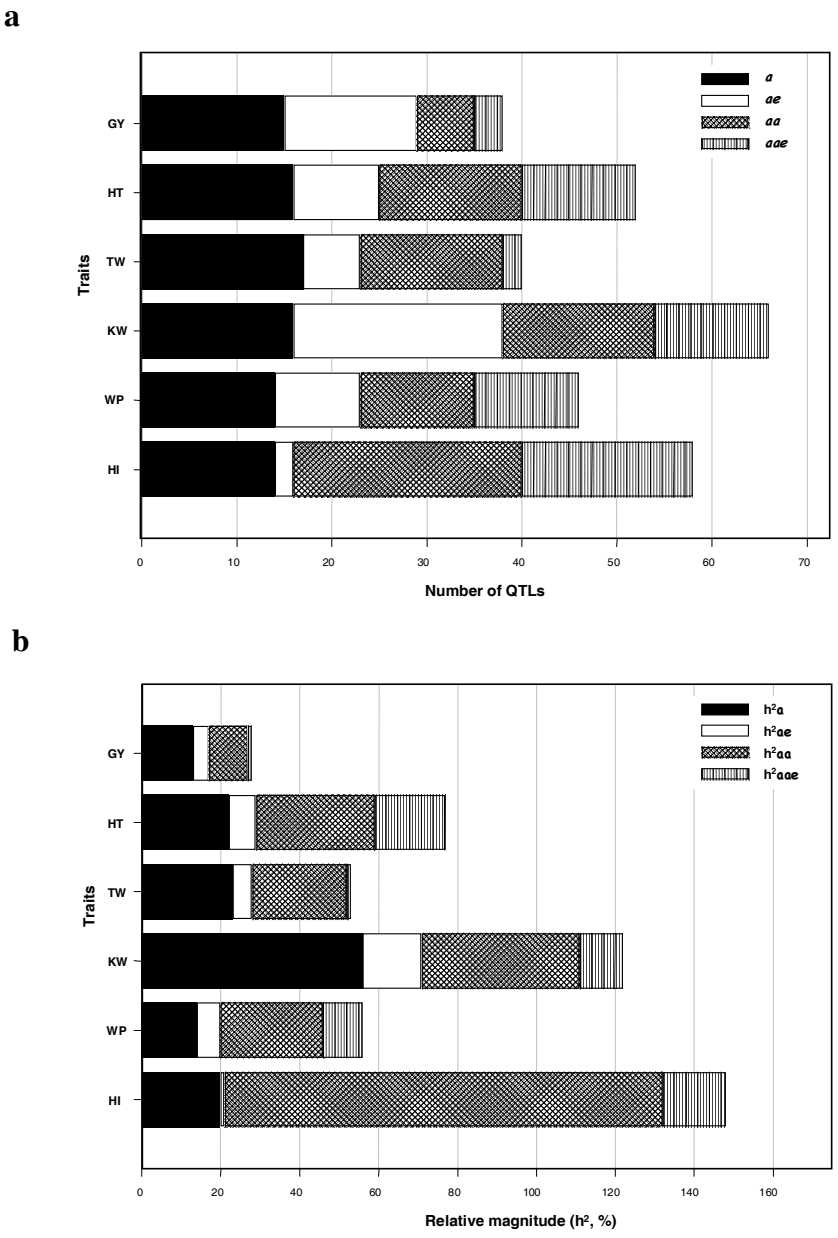
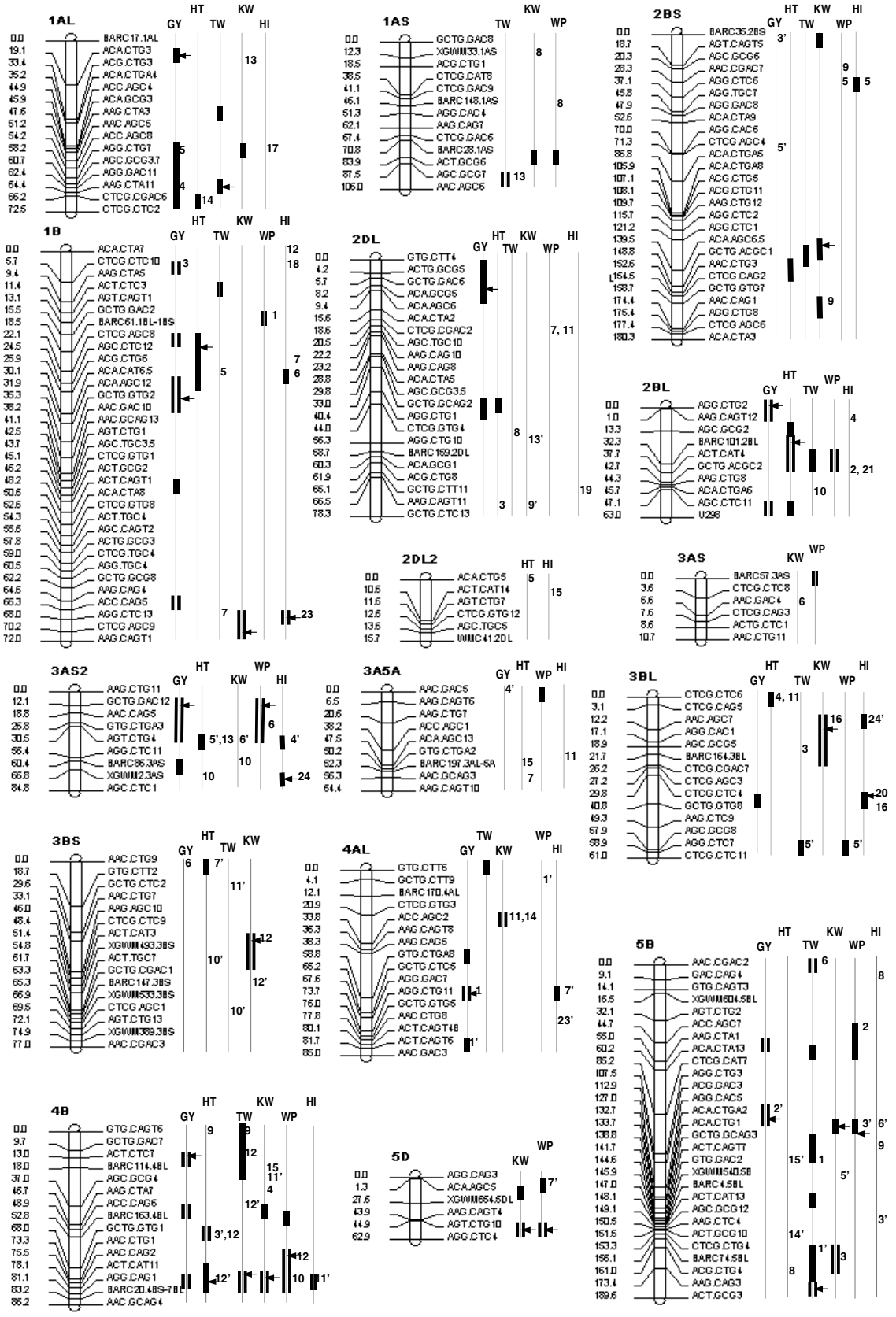


Fig. 1. Frequency distributions for genetic and non-genetic components of phenotypic variation for grain yield (GY), plant height (HT), test weight (TW), kernel weight (KW), wheat protein content (WP), and hardness index (HI) in the Ning7840 x Clark population: a) total number of significant ($P < 0.005$) QTLs identified for additive (*a*), additive x environment (*ae*), epistatic (*aa*), and epistatic x environment interaction (*aae*) effects, and b) total relative magnitude of significant ($P < 0.005$) *a*, *ae*, *aa*, and *aae* effects.


1AL
1AS
2BS
1B
2DL
2BL
2DL2
3AS
3AS2
3A5A
3BL
3BS
4AL
5B
4B
5D

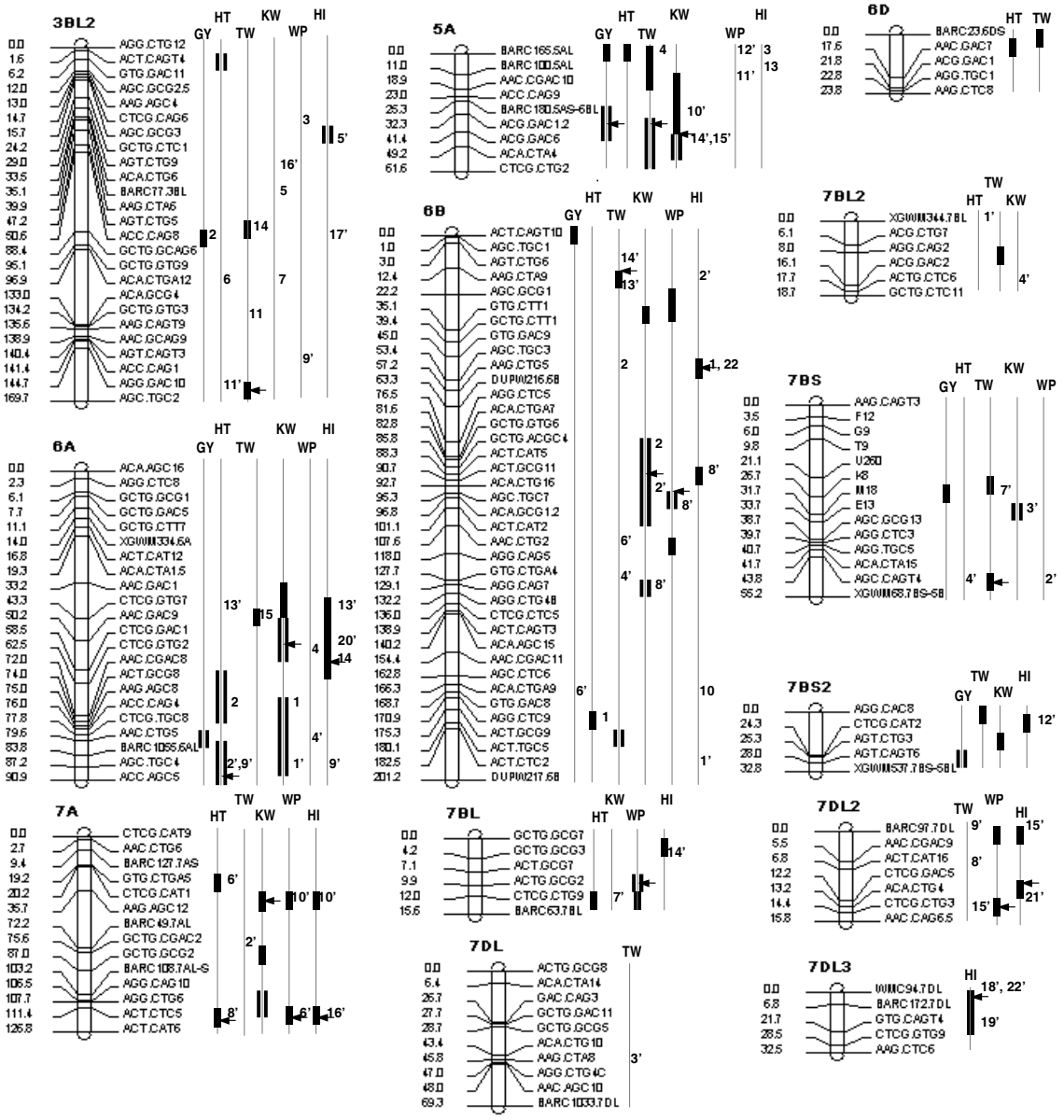


Fig. 2. Primary genomic regions of identified QTLs ($P < 0.005$) affecting grain yield (GY), plant height (HT), test weight (TW), kernel weight (KW), wheat protein content (WP), and hardness index (HI) in the Ning7840 x Clark RIL population evaluated in various Oklahoma environments from 2001 to 2003. A single and double bar indicate additive and additive x environment interaction, respectively. Arrows represent the interval exhibiting peak h^2. Intervals exhibiting additive x additive epistatic effects are ranked independently for each trait in pairs (e.g., 4 and 4' represent the pair of QTLs exhibiting digenic epistatic interaction in linkage groups 1AL and 3A5A with the fourth highest relative magnitude for grain yield).

VITA

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Doctor of Philosophy

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Scope and Methods of Study: Agronomic and quality traits are important factors in wheat (*Triticum aestivum* L.) improvement and in determining end-use product value. Knowledge regarding the number, genomic location, and effect of quantitative trait loci (QTL) would facilitate marker-assisted selection and the development of cultivars with desired trait complexes. Our objectives were to identify QTLs influencing agronomic and milling performance, and to determine their genetic effects. A population of 132 F_{12} recombinant inbred lines (RILs) was derived by single-seed descent from a cross between the Chinese facultative wheat, Ning7840, and the U.S. soft red winter wheat, Clark. The population was grown at three Oklahoma locations from 2001 to 2003. Measurements were collected for yield, yield components, plant adaptation, spike morphology, kernel size, and class factors.

Findings and Conclusions: Twenty-nine linkage groups, consisting of 363 AFLP and 47 SSR markers, were identified. Using composite interval mapping (CIM) analysis, 10, 16, 30, and 14 QTLs were detected for yield, yield components, plant adaptation, and spike morphology traits, respectively. Alleles from Clark were associated with a positive effect for the majority of QTLs for yield and yield components. Consistent, co-localized QTLs for yield and yield components were identified in linkage groups 1AL, 1B, 4B, 5A, 6A, and 7A, and less consistent but unique QTLs were found on 2BL, 2BS, 2DL, and 6B. For quality traits, a unique QTL was identified for test weight in linkage group 5B, presumably influencing grain packing efficiency. Common markers were identified for test weight, kernel weight, and kernel diameter on 5A. Consistent co-localized QTLs were identified for kernel weight and kernel diameter in linkage group 6A. Important QTLs with strictly additive effects were identified in linkage groups 5A (yield), 5A, 2BS (test weight), and 5B (kernel weight) through mixed-model QTL analysis. Epistatic QTLs tended to show a greater level of QTL x environment interaction than additive QTLs, suggesting that epistatic QTLs are more prone to environmental influence than additive QTLs. Results of this study provide a benchmark for future efforts on QTL identification.

ADVISER'S APPROVAL: Dr. Brett F. Carver