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ORIGINAL PAPER

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# Quantitative trait loci for yield and related traits in the wheat population Ning7840 × 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. 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.

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## Introduction

As the world's most important food crop, wheat (*Triticum aestivum* L.) grows on over 208 million hectares and now produces 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 and highly influenced by environmental conditions (Kearsey and Pooni 1996). These factors make it difficult to define yield according to gene effect and/or gene number using classical quantitative genetic methods. The application of new molecular marker technologies for quantitative trait locus (QTL) analysis has provided an effective approach to dissect complicated quantitative traits into component loci to study their relative effects on a specific trait (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: $2n = 6\times = 42$) and a relatively large genome size of 16,000 Mbp, of which more than 80% is repetitive DNA (Röder et al. 1998; Marshall et al. 2001). One advantageous marker class for QTL detection in wheat is represented by amplified fragment length polymorphism (AFLP) markers, which 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 microsatellite, which is stable, abundantly dispersed throughout the genome, and locus-specific in hexaploid wheat. Detailed SSR genetic maps are now available for wheat (Röder et al. 1998, 2002; Pestsova et al. 2000; Somers et al. 2004; Song et al. 2005). A 'skeletal' genetic map with SSRs can provide 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, (2) determine the chromosome locations and phenotypic effects of these yield-related QTLs, and (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. A two-row 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 October to early November), and fertilizer was added according to soil-test recommendations for a 4,000 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 per spike (KS) and kernel weight per spike, hereafter called spike weight (SW), were determined from the mean of 15 random spikes. Grain weight was reported at 11% relative humidity. 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 as no symptoms) to 5 (yellow canopy as 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 levels 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 2–4-week-old greenhouse-grown plants of both parents (Ning7840 and Clark) and the 132 $F_{12}$ RILs using the CTAB procedure (Saghai-Maroof et al. 1984). The PCR was performed in a volume of 12 µl containing 0.2 mM of each dNTP, 1× PCR buffer, 3 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

**Table 1** Phenotypic summary of yield-related traits, plant adaptation traits, and spike morphology for Ning7840, Clark, and their RIL progenies evaluated in three Oklahoma environments from 2001 to 2003

| Trait | Parents | | RIL population[a] | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Clark | Ning7840 | Mean | Max. | Min. | SD | $h^2$ | Multiple $R^2$ |
| Yield | | | | | | | | |
| Grain yield (kg ha$^{-1}$) | 2,595 | 2,219 | 2,328 | 4,058 | 872 | 655 | 0.83 | 0.89 |
| Spike number (m$^2$) | 589 | 517 | 525 | 823 | 329 | 85 | 0.49 | 0.77 |
| Kernel number (spike$^{-1}$) | 35.8 | 32.5 | 33.4 | 46.7 | 21.6 | 4.8 | 0.87 | 0.82 |
| Spike weight (g) | 1.10 | 0.81 | 0.93 | 1.28 | 0.58 | 0.13 | 0.70 | 0.83 |
| Plant adaptation | | | | | | | | |
| Heading date[b] (days) | 23 | 26 | 25 | 32 | 19 | 3.0 | 0.78 | 0.85 |
| Maturity date (1–4)[c] | 1.6 | 1.6 | 1.9 | 4.0 | 1.0 | 0.8 | 0.85 | 0.80 |
| Plant height (cm) | 80 | 76 | 78 | 97 | 58 | 8 | 0.90 | 0.91 |
| Shattering score (1–5)[d] | 2.1 | 2.0 | 2.0 | 4.2 | 1.0 | 0.9 | 0.61 | 0.58 |
| Lodging score (1–5)[e] | 1.2 | 1.3 | 1.9 | 4.3 | 1.0 | 0.8 | 0.56 | 0.70 |
| Leaf yellowing (1–5)[f] | 1.5 | 2.1 | 2.0 | 3.9 | 1.0 | 0.6 | 0.60 | 0.64 |
| Leaf rust reaction (%)[g] | 24.3 | 8.7 | 34.5 | 74.1 | 1.6 | 21.8 | 0.71 | 0.95 |
| Spike morphology | | | | | | | | |
| Spike length (cm) | 8.0 | 7.0 | 8.0 | 10.0 | 6.0 | 1.0 | 0.89 | 0.72 |
| Spike density (1–4)[h] | 3.3 | 2.4 | 3.0 | 4.0 | 1.0 | 0.7 | 0.87 | 0.88 |

[a]Population of 132 $F_{12}$ recombinant inbred lines
[b]Days after 31 March
[c]Early = 1, late = 4
[d]No shattering = 1, severe shattering = 5
[e]No lodging = 1, severe lodging = 5
[f]No yellowing = 1, severe yellowing = 5
[g]% Severity
[h]Compact = 1, lax = 4

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, USA) using a fluorescence-labeled M13 primer for PCR detection. The SSRs screened in this study included 181 XGWMs (Röder et al. 1998), 160 BARCs (Song et al. 2005), 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, multiple $R^2$ value and heritability ($h^2$) on a line-mean basis were calculated for all traits across environments using SAS (SAS Institute 2003).

## Quantitative trait locus 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. QTL was claimed to be significant at a LOD value of 3. 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

### 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 ten recombinations per 100 meiotic events, or an interval length less than 10 cM (Doerge 2002), therefore the map is suitable for genome-wide QTL scanning in this study.

### Phenotypic summary

The phenotypic data were classified into three categories: yield traits, plant adaptation traits, and spike morphology traits (Table 1). The ANOVA (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 (data not shown), 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 per spike, and spike weight were 9–26% 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 significantly for these traits, except for leaf rust.

Positive phenotypic correlation coefficients were found between each of the three yield components and grain yield (Fig. 2). Spike weight showed the strongest positive association with grain yield, which might be expected considering that spike weight integrates the effects of kernel number per spike 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. In addition, greater shattering, lodging, plant yellowing, and leaf rust susceptibility were associated with lower yield as expected. Hence, identification of QTLs with direct effects on yield requires scanning for QTLs that influence yield independently of these adaptation traits. Differences in spike density did not correlate with differences in grain yield, although more compact spikes made shorter spikes.

### Quantitative trait locus mapping

The composite-interval mapping analysis produced a total of 206 putative 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 due to lack of polymorphic SSRs identified in these chromosomes.

Ten QTL were identified for grain yield and each of them explained 7.3–21.1% phenotypic variation (Table 2, Fig. 1). For three yield components, one QTL on 3BS was found for spike number, eight QTLs were identified for kernel number and seven QTLs were detected for spike weight. For plant adaptation traits, three QTLs each were detected for heading and maturity date, lodging score, and leaf rust reaction; and six QTLs each were detected for plant height, shattering and leaf yellowing. In addition, ten QTLs were identified for spike length and four QTLs were identified for spike density. Overall, we detected a mean of six putative QTL for yield-related traits, four for plant adaptation traits, and seven for spike-morphology traits. These results are consistent 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).

## Discussion

### Quantitative trait loci for plant adaptation traits

Plant heading date, maturity date, height, leaf yellowing, leaf rust reaction, and shattering and lodging scores are considered as plant adaptation traits. 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 (Table 2). 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 accounted for 56% of the phenotypic variance (Table 2). Interestingly, this major QTL was the 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.

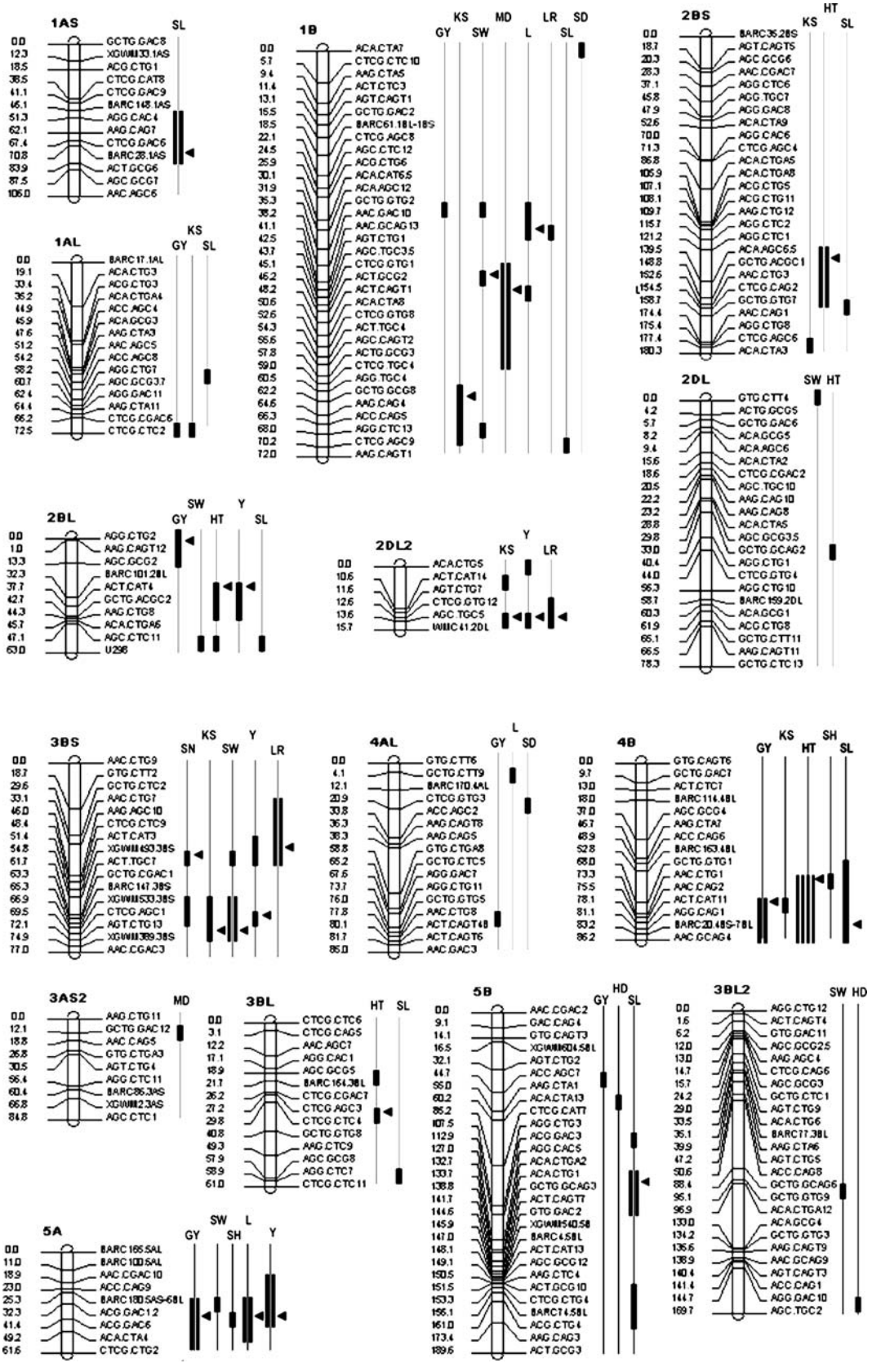


**Fig. 1** Primary genomic regions of QTLs identified by composite interval mapping for grain yield and yield components, plant adaptation traits, and spike morphology from the Ning7840 × Clark RIL population evaluated in Oklahoma from 2001 to 2003. *Bars* indicate the number of environments for which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

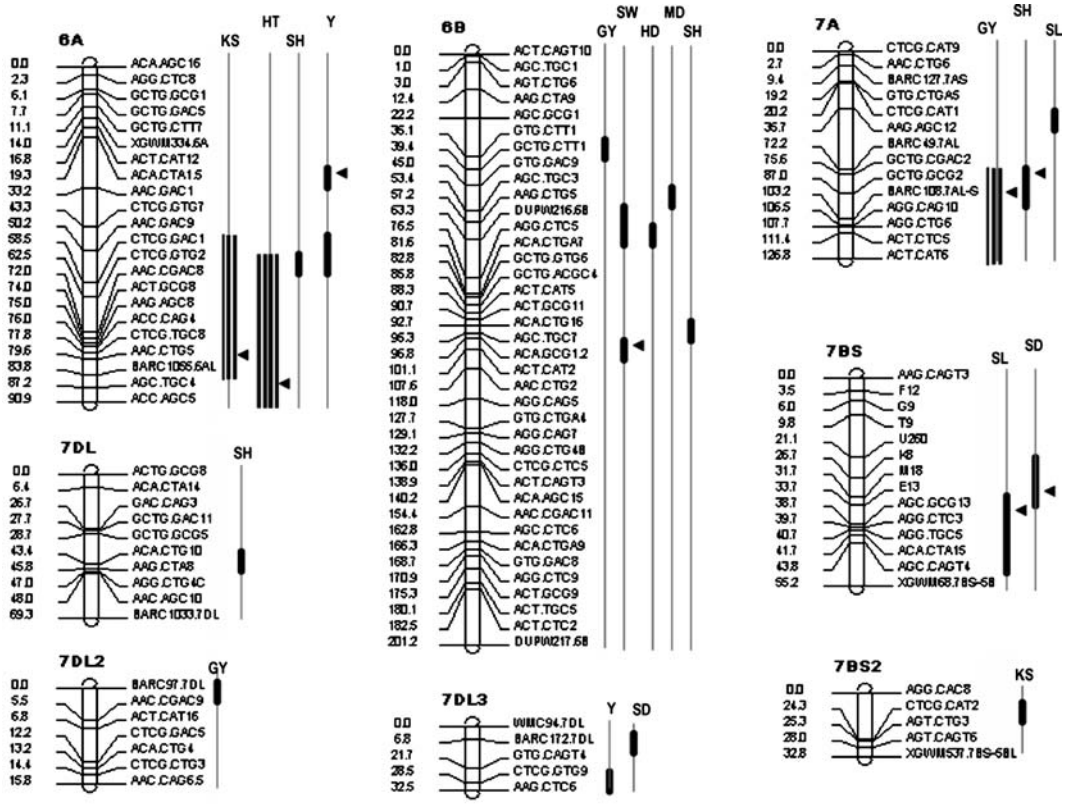


**Fig. 1** (Contd.)

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, Table 2). The only linkage group to which shattering was mapped in multiple environments was 6B, a linkage group relatively unimportant to direct expression of grain yield in this population. We have found no published precedent for a shattering QTL in wheat.

Three QTLs for lodging score were identified in linkage groups 1B, 4AL, and 5A. The QTL in 5A was identified in two of three environments. Keller et al. (1999) reported a QTL in the similar location. Chromosome 5A is also mentioned as one of the locations of a stem solidness gene (Cook et al. 2004). Among all correlated traits plotted in Fig. 2, lodging score showed the strongest negative association with yield. This relationship may in part be attributed to the consistent QTL on linkage groups 5A and 1B, which mapped to the same chromosome region for both traits. For these regions, the alleles 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. 2002) based on microsatellite marker *XGWM37* that was also located on 7DL. A single QTL was identified 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 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 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. The linkage group 6B harboring QTL for heading date also influenced maturity date. Two QTLs unique to

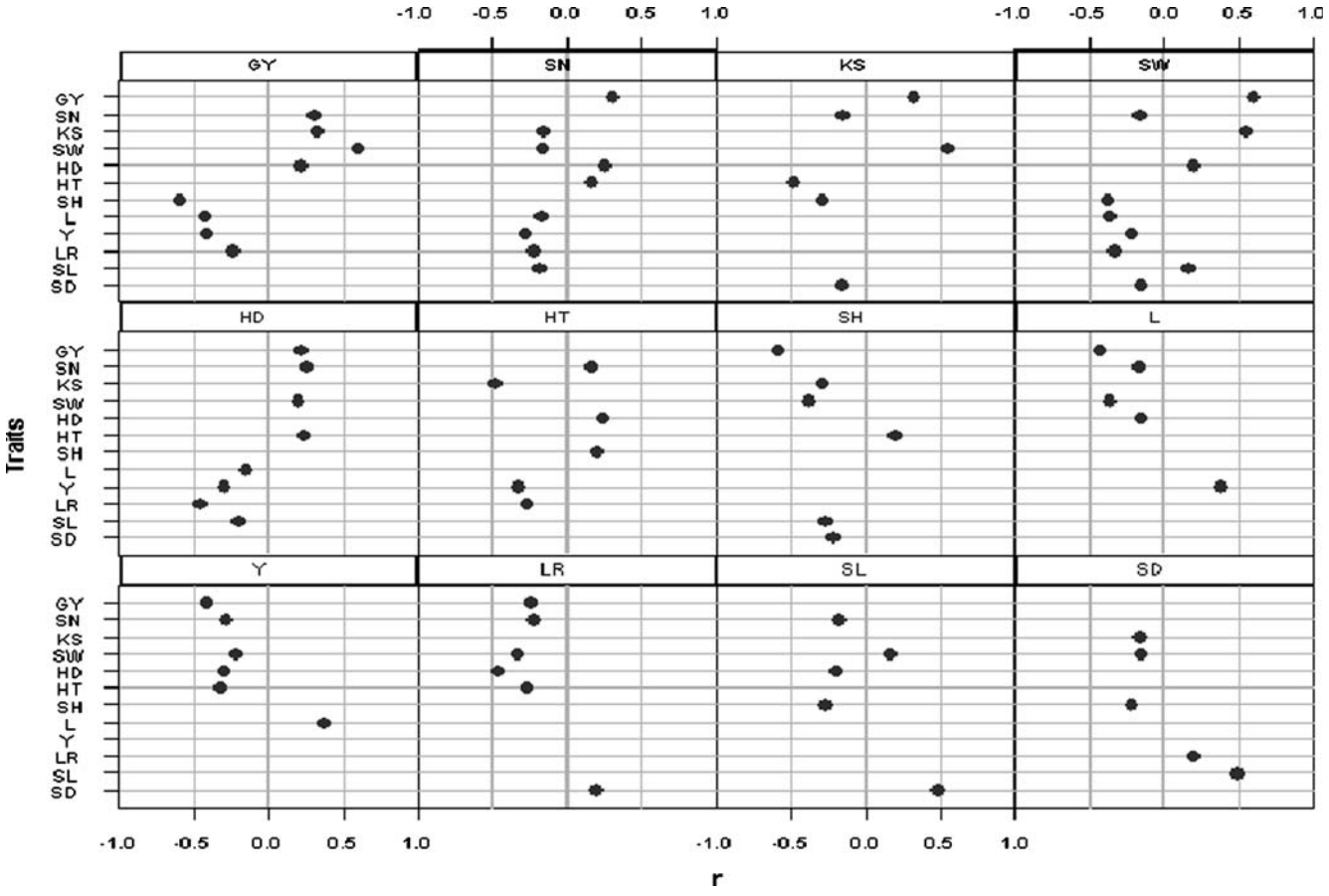


**Fig. 2** Correlation coefficient plot among yield-related traits, plant adaptation traits, and spike morphology for the Ning7840 × Clark RIL population evaluated in Oklahoma from 2001 to 2003. Only significant *r*-values ($P < 0.05$) are shown in the plot. Traits are grain yield (*GY*), spike number (*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*)

maturity date were detected in linkage groups 1B and 3AS2, 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. We found no significant association between yield and height in this population to warrant the consideration of height QTLs to indirectly manipulate yield (Figs. 1, 2). However, a common marker interval was identified in linkage group 4B (*ACT.CAT11*//*AAC.GCAG4*; Table 2), in which the allele from Clark increased yield but decreased plant height.

## Quantitative trait loci for spike morphology

Ten 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 (*AGC.GCG13*/*AGG.CT3*) associated with both traits (Fig. 1).

## Quantitative trait loci for yield traits

Ten QTLs were detected for grain yield (Table 2) 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, accounting for 8–19% of the phenotypic variation. Alleles from Ning7840 increased yield at the other five QTLs in linkage groups 1AL, 1B, 5B, 7A, and 7DL2, accounting for 9–21% of the phenotypic variance.

**Table 2** Primary genomic regions (consistent in dark underlined), environments (Lahoma 2002 and 2003, LA03 and LA02; Stillwater 2001, 2002, and 2003, ST01, ST02, and ST03; and Altus 2002 and 2003, AL02 and AL03, respectively), and their associated additive gene effects for grain yield-related traits, plant adaptation traits, and spike morphology identified by composite interval mapping (CIM)

| Linkage group | Environment | Position (cM) | Marker interval | LOD[a] | $a$[b] | $R^2$(%) |
|---|---|---|---|---|---|---|
| Grain yield | | | | | | |
| Grain yield (kg ha$^{-1}$) | | | | | | |
| 1AL | LA03 | 66 | *CTCG.CGAC6/CTCG.CTC2* | 3.2 | −252 | 9.4 |
| 1B | ST03 | 35 | *GCTG.GTG2/AAC.GAC10* | 3.4 | −172 | 9.6 |
| 2BL | LA03, ST01, AL03 | 3 | *AAG.CAGT12/AGC.GCG2* | 3.5 | 253 | 11.3 |
| 4AL | ST01, | 78 | *AAC.CTG8/ACT.CAGT4B* | 3.2 | 181 | 7.6 |
| 4B | ST03, ST02, LA02 | 78 | *ACT.CAT11/AGG.CAG1* | 4.0 | 267 | 10.2 |
| **5A** | LA03, ST01, AL02, ST02, LA02, AL03 | 38 | *ACG.GAC1.2/ACG.GAC6* | 6.0 | 241 | 18.5 |
| 5B | ST03, ST02 | 49 | *ACC.AGC7/AAG.CTA1* | 3.1 | −185 | 11.2 |
| 6B | ST03, ST01, LA02 | 39 | *GCTG.CTT1/GTG.GAC9* | 3.1 | 175 | 7.3 |
| 7A | ST03, AL02, ST02, AL03 | 103 | *BARC108.7AL-S/AGG.CAG10* | 7.0 | −361 | 21.1 |
| 7DL2 | AL03 | 4 | *BARC97.7DL/AAC.CGAC9* | 3.3 | −384 | 10.6 |
| Spike number (No. m$^{-2}$) | | | | | | |
| 3BS | ST01 | 59 | *XGWM493.3BS/ACT.TGC7* | 4.3 | −25 | 12.0 |
| Kernel number (No. Spike$^{-1}$) | | | | | | |
| 1AL | LA03, ST02 | 68 | *CTCG.CGAC6/CTCG.CTC2* | 3.6 | 1.2 | 9.0 |
| 1B | ST01 | 62 | *GCTG.GCG8/AAG.CAG4* | 5.1 | 1.9 | 12.0 |
| 2BS | LA03, ST03 | 179 | *CTCG.AGC6/ACA.CTA3* | 3.3 | 1.2 | 9.3 |
| 2DL2 | LA03 | 14 | *AGC.TGC5/WMC41.2DL* | 4.5 | −1.3 | 12.2 |
| 3BS | AL02, ST02 | 72 | *AGT.CTG13/XGWM389.3BS* | 3.9 | 1.2 | 8.7 |
| 4B | LA03, ST03, AL02, ST02 | 78 | *ACT.CAT11/AGG.CAG1* | 6.0 | 1.5 | 14.1 |
| **6A** | LA03, ST03, ST01, AL02, ST02 | 80 | *AAC.CTG5/AAC.CTG5* | 7.4 | −2.1 | 21.0 |
| 7BS2 | ST01 | 24 | *CTCG.CAT2/AGT.CTG3* | 4.1 | 1.7 | 9.6 |
| Spike weight (g) | | | | | | |
| 1B | ST03, AL02 | 46 | *ACT.GCG2/ACT.CAGT1* | 3.5 | −0.06 | 7.9 |
| 2BL | LA03 | 61 | *AGC.CTC11/U298* | 3.1 | −0.04 | 11.0 |
| 2DL | ST02 | 0 | *GTG.CTT4/ACTG.GCG5* | 4.1 | 0.05 | 9.7 |
| 3BL2 | LA03 | 88 | *GCTG.GCAG6/GCTG.GTG9* | 3.2 | −0.04 | 9.9 |
| 3BS | AL02, ST02 | 72 | *AGT.CTG13/XGWM389.3BS* | 4.8 | 0.04 | 11.3 |
| 5A | AL02 | 25 | *BARC180.5AS-6BL/ACG.GAC1.2* | 4.7 | 0.06 | 10.7 |
| **6B** | LA03, ST01, AL02 | 95 | *AGC.TGC7/ACA.GCG1.2* | 4.5 | 0.06 | 13.2 |
| Plant adaptation | | | | | | |
| Heading date (days) | | | | | | |
| 3BL2 | LA03, LA02 | 169 | *AGG.GAC10/AGC.TGC2* | 3.3 | −1.1 | 9.3 |
| **5B** | ST03, ST02, LA02 | 60 | *ACA.CTA13/CTCG.CAT7* | 4.7 | −1.1 | 12.0 |
| 6B | ST03 | 77 | *AGG.CTC5/ACA.CTGA7* | 3.4 | −0.9 | 10.7 |
| Maturity date rating (1–4) | | | | | | |
| **1B** | ST03, ST01 | 50 | *ACT.CAGT1/ACA.CTA8* | 3.9 | −0.27 | 9.9 |
| 3AS2 | ST03 | 14 | *GCTG.GAC12/AAC.CAG5* | 3.3 | 0.28 | 10.0 |
| 6B | ST03 | 57 | *AAG.CTG5/DUPW216.6B* | 4.2 | −0.32 | 11.5 |
| Plant height (cm) | | | | | | |
| 2BL | ST03, ST01 | 40 | *ACT.CAT4/GCTG.ACGC2* | 6.0 | 3.0 | 16.7 |
| 2BS | ST02, LA02 | 144 | *ACA.AGC6.5/GCTG.ACGC1* | 6.0 | −3.8 | 16.9 |
| 2DL | ST01 | 33 | *GCTG.GCAG2/AGG.CTG1* | 4.9 | 2.8 | 12.3 |
| 3BL | ST02, LA02 | 27 | *CTCG.AGC3/CTCG.CTC4* | 4.4 | 2.9 | 9.6 |
| **4B** | ST03, ST01, LA02 | 75 | *AAC.CTG1/AAC.CAG2* | 6.7 | −2.8 | 14.9 |
| **6A** | LA03, ST03 | 87 | *AGC.TGC4/ACC.AGC5* | 5.6 | 2.5 | 12.1 |
| Shattering score (1–5) | | | | | | |
| 4B | ST02, AL03 | 73 | *AAC.CTG1/AAC.CAG2* | 3.5 | −0.21 | 9.2 |
| 5A | LA02 | 32 | *ACG.GAC1.2/ACG.GAC6* | 3.3 | −0.36 | 8.9 |
| 6A | AL03 | 63 | *CTCG.GTG2/AAC.CGAC8* | 3.2 | 0.50 | 9.3 |
| **6B** | LA03, ST03, ST02 | 93 | *ACA.CTG16/AGC.TGC7* | 3.3 | 0.84 | 10.2 |
| 7A | ST02, AL03 | 99 | *GCTG.GCG2/BARC108.7AL-S* | 3.3 | 0.59 | 12.1 |
| 7DL | ST03 | 56 | *AAC.AGC10/AAG.CTA8* | 9.8 | −0.61 | 55.9 |
| Lodging score (1–5) | | | | | | |
| 1B | ST02 | 41 | *AAC.GCAG13/AGT.CTG1* | 7.1 | 0.37 | 16.7 |
| 4AL | LA03 | 4 | *GCTG.CTT9/BARC170.4AL* | 5.0 | 0.36 | 14.1 |
| **5A** | LA03, ST03 | 38 | *ACG.GAC1.2/ACG.GAC6* | 5.9 | −0.39 | 23.0 |

**Table 2** (Contd.)

| Linkage group | Environment | Position (cM) | Marker interval | LOD[a] | $a$[b] | $R^2$(%) |
|---|---|---|---|---|---|---|
| Leaf yellowing (1–5) | | | | | | |
| 2BL | LA02 | 38 | *ACT.CAT4/GCTG.ACGC2* | 4.6 | −0.29 | 11.0 |
| 2DL2 | LA03 | 16 | *AGC.TGC5/WMC41.2DL* | 5.2 | 0.31 | 14.5 |
| 3BS | LA02 | 72 | *CTCG.AGC1/AGT.CTG13* | 4.0 | 0.27 | 9.3 |
| **5A** | LA03, ST03, LA02 | 38 | *ACG.GAC1.2/ACG.GAC6* | 6.0 | −0.35 | 16.6 |
| 6A | LA03, ST03 | 31 | *ACA.CTA1.5/AAC.GAC1* | 4.3 | −0.35 | 12.3 |
| 7DL3 | LA03 | 29 | *CTCG.GTG9/AAG.CTC6* | 3.4 | −0.26 | 8.7 |
| Leaf rust reaction (%) | | | | | | |
| 1B | ST02, LA02 | 41 | *AAC.GCAG13/AGT.CTG1* | 3.4 | 7.7 | 7.4 |
| 2DL2 | ST02, LA02 | 16 | *AGC.TGC5/WMC41.2DL* | 3.5 | −9.2 | 7.9 |
| **3BS** | ST02, LA02 | 51 | *ACT.CAT3/XGWM493.3BS* | 7.2 | −11.6 | 16.9 |
| Spike morphology | | | | | | |
| Spike length (cm) | | | | | | |
| 1AL | LA03 | 58 | *AGG.CTG7/AGC.GCG3.7* | 4.1 | −0.44 | 12.8 |
| 1AS | ST03, AL02 | 79 | *BARC28.1AS/AGT.GCG6* | 3.3 | 0.31 | 10.8 |
| 1B | ST03, ST02 | 70 | *CTCG.AGC9/AAG.CAGT1* | 3.7 | −0.30 | 9.6 |
| 2BL | ST01 | 53 | *AGC.CTC11/U298* | 3.8 | 0.37 | 11.9 |
| 2BS | LA03, ST01 | 159 | *GCTG.GTG7/AAC.CAG1* | 4.3 | 0.31 | 13.7 |
| **3BL** | LA03, ST01, AL02, ST02 | 61 | *AGG.CTC7/CTCG.CTC11* | 3.3 | −0.30 | 7.4 |
| 4B | ST02 | 83 | *BARC20.4BS-7BL/AAC.GCAG4* | 8.2 | 0.40 | 18.0 |
| 5B | LA03, ST03, ST01, AL02 | 134 | *ACA.CTG1/GCTG.GCAG3* | 6.8 | −0.44 | 16.6 |
| 7A | ST03, ST02 | 24 | *CTCG.CAT1/AAG.AGC12* | 4.7 | 0.40 | 17.1 |
| 7BS | ST02 | 39 | *AGC.GCG13/AGG.CTC3* | 4.3 | −0.28 | 8.7 |
| Spike density (1–4) | | | | | | |
| **1B** | LA03, ST03 | 0 | *ACA.CTA7/CTCG.CTC10* | 3.2 | −0.24 | 9.8 |
| 4AL | LA03 | 21 | *CTCG.GTG3/ACC.AGC2* | 3.5 | 0.22 | 11.8 |
| 7BS | ST01 | 38 | *E13/AGC.GCG13* | 5.8 | −0.33 | 15.9 |
| 7DL3 | ST03 | 7 | *BARC172.7DL/GTG.CAGT4* | 3.8 | −0.29 | 14.1 |

[a]LOD value was calculated based on line mean from each location. All locations listed have a LOD value of 3 or higher. The largest LOD value was listed if more than one location showed a LOD value of 3 or higher

[b]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

Chromosome 5A, where our most repeatable yield QTL was identified, is known to carry a number of major genes affecting anthesis date, frost tolerance, drought tolerance (Sourdille 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 resulted in no common QTLs between them (Figs. 1, 2). Inconsistent parental differences in spike number (data not shown) further hindered an attempt to detect meaningful QTLs for this yield component. Linkage group 3BS contained a QTL for spike number that explained 12% of the phenotypic variance (Table 2). 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.

In contrast to spike number, eight QTLs were detected for kernel number per spike (Table 2). Six of these were mapped to linkage groups 1AL, 1B, 2BS, 3BS, 4B, and 7BS2 at which the Clark allele increased kernel number per spike. 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 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 per spike, *CTCG.CGAC6/CTCG.CT2* on 1AL and *ACT.CAT11/AGG.CAG1* on 4B, showed common effects on grain yield in some, but not all, environments (Table 2, Fig. 1).

Distinct differences between parental lines for spike weight allowed the identification of seven QTLs in as many linkage groups (Table 2). Four QTLs in linkage groups 2DL, 3BS, 5A, and 6B explained 10–13% 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 8–11% 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 on chromosomes 3BS and 6A (Huang et al. 2004), 4A (Araki et al. 1999; Borner et al. 2002), and 5A (Kato et al. 2000). The strongest phenotypic association exhibited by spike weight and yield (Fig. 2) may be reflected in the common QTL region in 1B and 5A. 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, 6B, and 7A. Grain yield QTLs were mapped in the same positions as that for kernel number on linkage groups 1A and 4B and for spike weight on 1B and 5A, suggesting that kernel size and spike weight may directly contribute to yield in those four genomic regions. No QTL for spike number was mapped in a yield QTL region. Considering all traits (Table 2), a QTL for spike number, kernel number per spike, and spike weight mapped to the same position in the marker interval *XGWM533*/*CTCG.AGC1* (3BS) as did a QTL for kernel number per spike and kernel weight in the marker interval *AGG.CTC13*/*CTCG.AGC9* (1B) and *AGT.CTG13*/*XGWM389* (3BS).

In addition, genomic regions significantly associated with traits conditioning adaptation were also associated with yield. 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 QTL with pleiotropic effects or that the genomic regions associated with these QTLs harbor a cluster of linked genes associated with yield potential and adaptation.

Summarizing across all traits, the QTLs for an unusually high number of traits were located on the linkage group 1B (8 from 13 possible, Fig. 1). Ning7840 is believed to possess the 1RS.1BL translocation (NGRP 2005), which was likely segregating in this RIL population. The 1RS.1BL translocation from Avrora was previously shown to increase grain yield in Oklahoma by 9–10% (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 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 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 flanking 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 for 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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## References

Araki E, Miura H, Sawada S (1999) Identification of genetic loci affecting amylose content and agronomic traits on chromosome 4A of wheat. Theor Appl Genet 98:977–984

Bai G, Kolb FL, Shaner G, Domier LL (1999) Amplified fragment length polymorphism markers linked to a major quantitative trait locus controlling scab resistance in wheat. Phytopatho 89:343–348

Basten CJ, Weir BS, Zeng Z-B (2001) QTL cartographer: a reference manual and tutorial for QTL mapping. Department of Statistics, North Carolina State University, Raleigh, pp 55–72

Borner A, Schumann E, Furste A, Coster H, Leithold B, Röder MS, Weber WE (2002) Mapping of quantitative trait loci determining agronomic important characters in hexaploid wheat (*Triticum aestivum* L). Theor Appl Genet 105:921–936

Bullrich L, Appendino ML, Tranquilli G, Lewis S, Dubcovsky J (2002) Mapping of a thermo-sensitive earliness *per se* gene on *triticum monococcum* chromosome $1A^m$. Theor Appl Genet 105:585–593

Cadalen T, Sourdille P, Charmet G, Tixier MH, Gay G, Boeuf C, Bernard S, Leroy P, Bernard M (1998) Molecular markers linked to genes affecting plant height in wheat using a double-haploid population. Theor Appl Genet 96:933–940

Campbell BT, Baenziger PS, Gill KS, Eskridge KM, Budak H, Erayman M, Dweikat I, Yen Y (2003) Identification of QTLs and environmental interactions associated with agronomic traits on chromosome 3A of wheat. Crop Sci 43:1493–1505

Carver BF, Rayburn AL (1994) Comparison of related wheat stocks possessing 1B or 1BL1RS chromosomes: agronomic performance. Crop Sci 34:1505–1510

Cook JP, Wichman DM, Martin JM, Bruckner PL, Talbert LE (2004) Identification of microsatellite markers associated with a stem solidness locus in wheat. Crop Sci 44:1397–1402

Doerge RW (2002) Mapping and analysis of quantitative trait loci in experimental populations. Nat Genet 3:43–52

Food and Agricultural Organization (2004) FAO, Rome. http://www.fao.org/ag/

Gupta K, Balyan S, Edwards J, Isaac P, Korzun V, Röder M, Gautier MF, Joudrier P, Schlatter R, Dubcovsky J, De La Pena C, Khairallah M, Penner G, Hayden J, Sharp P, Keller B, Wang C, Hardouin P, Jack P, Leroy P (2002) Genetic mapping of 66 new microsatellite (SSR) loci in bread wheat. Theor Appl Genet 105:213–422

Henry M, Ginkel MV, Khairallah M (2002) Marker-assisted selection for BYDV resistance in wheat. Mexico, DF, Mexico, pp 41–44. http://www.cimmyt.org/Research

Huang XQ, Coster H, Ganal MW, Röder MS (2003) Advanced backcross QTL analysis for the identification of quantitative trait loci alleles from wild relatives of wheat (*Triticum aestivum* L). Theor Appl Genet 106:1379–1389

Huang XQ, Kempf H, Ganal MW, Röder MS (2004) Advanced backcross QTL analysis in progenies derived from a cross between a German elite winter wheat variety and a synthetic wheat (*Triticum aestivum* L). Theor Appl Genet 109:933–943

Kato K, Miura H, Sawada S (2000) Mapping QTLs controlling grain yield and its components on chromosome 5A of wheat. Theor Appl Genet 101:1114–1121

Kearsey MJ, Farquhar AGL (1998) QTL analysis in plants; where are we now? Heredity 80:137–142

Kearsey MJ, Pooni HS (1996) The genetical analysis of quantitative traits. 1st edn. Chapman and Hall, London

Keller M, Karutz Ch, Schmid JE, Stamp P, Winzeler M, Keller B, Messmer MM (1999) Quantitative trait loci for lodging resistance in a segregating wheat × spelt population. Theor Appl Genet 98:1171–1182

Kosambi DD (1944) The estimation of map distances from recombination values. Ann Eugen 12:172–175

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg I (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

Marshall DR, Langridge P, Appels R (2001) Wheat breeding in the new century. http://www.publish.csiro.au/journals/ajar

National Genetic Resources Program (2005) Germplasm resources information network. USDA-ARS, Beltsville. http://www.ars-grin.gov/cgi-bin/npgs/html

Ohm HW, Shaner G, Foster JE, Patterson FL, Buechley G (1988) Registration of 'Clark' wheat. Crop Sci 28:1031–1032

Pestsova E, Ganal MW, Roder MS (2000) Isolation and mapping of microsatellite markers specific for the D genome of bread wheat. Genome 43:689–697

Röder MS, Korsun V, Wendehake K, Plaschke J, Tixier MH, Leroy P, Ganal MW (1998) A microsatellite map of wheat. Genetics 149:2007–2023

Röder M, Wendehake K, Korzun V, Bredemeijer G, Laborie D, Bertrand L, Isaac P, Rendell S, Jackson J, Cooke R, Vosman B, Ganal M (2002) Construction and analysis of a microsatellite-based database of European wheat varieties. Theor Appl Genet 106:67–73

Saghai-Maroof MA, Soliman KM, Jorgensen RA, Allard RW (1984) Ribosomal DNA spacer-length polymorphism in barley: Mendelian inheritance, chromosome location, and population dynamics. Proc Natl Acad Sci USA 81:8014–8018

SAS Institute (2003) SAS/STAT user's guide, version 9.1. SAS Institute, Cary

Shah MM, Gill KS, Baenziger PS, Yen Y, Kaeppler SM, Ariyarathne HM (1999) Molecular mapping of loci for agronomic traits on chromosome 3A of bread wheat. Crop Sci 39:1728–1732

Shindo C, Tsujimoto H, Sasakuma T (2003) Segregation analysis of heading traits in hexaploid wheat utilizing recombinant inbred lines. Heredity 90:56–63

Singh RP, Nelson JC, Sorrells ME (2000) Mapping Yr28 and other genes for resistance to stripe rust in wheat. Crop Sci 40:1148–1155

Somers DJ, Isaac P, Edwards K (2004) A high-density microsatellite consensus map for bread wheat (*Triticum aestivum* L). Theor Appl Genet 109:1105–1114

Song QJ, Shi JR, Singh S, Fickus EW, Costa JM, Lewis J, Gill BS, Ward R, Cregan PB (2005) Development and mapping of microsatellite (SSR) markers in wheat. Theor Appl Genet 110:550–560

Sourdille P, Tixier MH, Charmet G, Gay G, Cadalen T, Bernard S, Bernard M (2000) Location of genes involved in ear compactness in wheat (*Triticum aestivum*) by means of molecular markers. Mol Breed 6:247–255

Sourdille P, Cadalen T, Gay G, Gill B, Bernard M (2002) Molecular and physical mapping of genes affecting awning in wheat. Plant Breed 121:320–324

Toth B, Galiba G, Feher E, Sutka J, Snape JW (2003) Mapping genes affecting flowering time and frost resistance on chromosome 5B of wheat. Theor Appl Genet 107:509–514

Vos P, Hogers R, Bleeker M, Reijans M, Van de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res 23:4407–4414

Wang S, Basten CJ, Zeng Z (2004) Windows QTL cartographer. V2.0 Program in statistical genetics, North Carolina State University, North Carolina. http://www.statgen.ncsu.edu/qtlcart/WQTLCart.htm