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Advanced backcross QTL analysis of a hard winter wheat × synthetic wheat population

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Abstract Advanced backcross quantitative trait locus (AB-QTL) analysis was used to identify QTLs for yield and yield components in a backcross population developed from a cross between hard red winter wheat (*Triticum aestivum* L.) variety Karl 92 and the synthetic wheat line TA 4152-4. Phenotypic data were collected for agronomic traits including heading date, plant height, kernels per spike, kernel weight, tiller number, biomass, harvest index, test weight, grain yield, protein content, and kernel hardness on 190 $BC_2F_{2:4}$ lines grown in three replications in two Kansas environments. Severity of wheat soilborne mosaic virus (WSBMV) reaction was evaluated at one location. The population was genotyped using 151 microsatellite markers. Of the ten putative QTLs identified, seven were located on homoeologous group 2 and group 3 chromosomes. The favorable allele was contributed by cultivated parent Karl 92 at seven QTLs including a major one for WSBMV resistance, and by the synthetic parent at three QTLs: for grain hardness, kernels per spike, and tiller number.

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Introduction

Bread wheat (*Triticum aestivum*. L.) is an allohexaploid (AABBDD) that arose from the hybridization of two species, tetraploid *T. turgidum* (AABB) and diploid *Aegilops tauschii* Cosson (DD) (Kihara 1944; McFadden and Sears 1946). Common wheat has been a genetically narrow species through out its entire existence due to the genetic bottlenecks associated with interspecific hybridization, polyploidization and selection (Cox 1998). As a result lower levels of polymorphism are observed for many traits in common wheat in comparison with its progenitor species (Ladizinsky 1985; Kam-Morgan et al. 1989). Broadening the genetic base of common wheat by identification and introgression of potentially useful genes from progenitor species is an important germplasm development goal.

Wide-crossing programs aimed at wheat improvement by gene transfer from wild species have focused mostly on traits such as pest resistance that are under simple genetic control. Most wild progenitor accessions are agronomically inferior to modern cultivars and even when they carry superior alleles for polygenic traits, linkage drag makes transfer by conventional backcrossing and selection impractically slow. However, there have been reports of improvements in traits such as protein concentration and kernel weight during introgression of genes from *Ae. tauschii* by direct crosses with *T. aestivum* (Cox et al. 1995; Fritz et al. 1995).

Synthetic hexaploid wheats, produced by interspecific hybridization of *T. turgidum* ($2n = 28$) and *Ae. tauschii* ($2n = 14$) followed by chromosome doubling, provide a straightforward way of transferring genes across ploidy levels, particularly from wild *Ae. tauschii* accessions. These amphiploids are a source of genes not only for pest resistance, but for traits with complex inheritance

(Mujeeb-Kazi 2000, 2001; Villareal et al. 2001). Genes introgressed from synthetic hexaploid wheat were found to improve quality and agronomic traits of spring wheat (del Blanco et al. 2000, 2001).

Advanced backcross QTL analysis (AB-QTL analysis) was proposed as a molecular-breeding method that integrates QTL analysis with germplasm development in crosses between adapted and wild germplasm (Tanksley and Nelson 1996). It relies on the partial isolation of wild QTLs by several backcrosses to an adapted parent to eliminate much of the wild genetic background. QTLs identified in this advanced material can then be isolated in near-isogenic lines (QTL-NILs) by further backcrossing and selfing. This approach can provide a means to tap the genetic diversity available in exotic germplasm and to isolate beneficial genes that are often discarded due to unfavorable linkages. Using the AB-QTL method, many QTLs have been identified and transferred from wild germplasm to elite breeding lines in crops such as tomato (Tanksley et al. 1996; Bernacchi et al. 1998; Fulton et al. 1997, 2000), pepper (Rao et al. 2003) rice (Xiao et al. 1998; Moncada et al. 2001; Septiningsih et al. 2003), maize (Ho et al. 2002), barley (Pillen et al. 2003) and wheat (Huang et al. 2003).

Microsatellite markers, also called simple sequence repeat (SSR) markers, are often multiallelic and chromosome specific and are distributed throughout the wheat genome (Röder et al. 1998a, b). The high levels of polymorphism detected between closely related species with SSR markers makes them ideal for tagging genes introgressed from wheat progenitors (Siedler et al. 1994; Plaschke et al. 1995; Joshi and Nguyen 1993).

We used wheat microsatellite markers to identify QTLs for agronomic traits in a BC_2F_2-derived population from a cross between *T. aestivum* and synthetic hexaploid wheat. The objectives were to assess the efficiency of AB-QTL analysis for identification of agronomically useful wheat QTLs and the performance of synthetic-derived QTL alleles in the target germplasm.

Materials and methods

Plant materials

A backcross population was developed using the hard red winter wheat cultivar 'Karl 92' as the recurrent and synthetic hexaploid wheat, TA 4152-4 (CIGM87.2775-1B-0PR-0B), as the donor parent. The synthetic hexaploid TA 4152-4 was selected based on vigor and plant type in the field at El Batan, Mexico by T.S. Cox. The line was reported to be resistant to stem rust and Karnal bunt and moderately susceptible to leaf rust when evaluated at Obregon and El Batan, Mexico (Mujeeb-Kazi et al. 2000). Karl 92 (PI-564245) is a semi-dwarf, early-maturing, hard red winter wheat with good breadmaking quality and resistance to WSBMV (Sears et al. 1997). The synthetic TA 4152-4 was developed from the cross between durum wheat *T. turgidum* cultivar 'Altar 84' and *Ae. tauschii* accession 'WX 193' and was kindly provided by the International Maize and Wheat Improvement Center (CIMMYT), Mexico.

Population development

TA 4152-4 was crossed as the female parent to Karl 92 and F_1 plants were then backcrossed as the female to Karl 92. Fifteen BC_1F_1 plants were again backcrossed to Karl 92 to produce at least 15–20 BC_2F_1 plants each. Thirty F_2 progeny from each of the 120 BC_2F_1 plants that survived, matured, and did not have glumes too tough to allow threshing were grown in the greenhouse and selection was applied against shattering and/or very tough glumes. From about 1,200 $BC_2F_{2:3}$ lines, 480 were selected in the greenhouse and grown in the field and 190 were selected for vigor, winter hardiness and threshability. These lines represent the progeny of 35 plants from 8 BC_2F_1 families tracing their parentage to just 3 of the original 15 BC_1 plants (Table 1).

Field trials

One hundred and ninety selected lines were tested for yield and related characters in harvest year 2003 at Manhattan and Hutchinson, Kansas. The backcross lines along with Karl 92 were sown in a randomized complete block design with three replicates. In each replicate, all entries were blocked by BC_2 family and each block comprised the lines tracing back to one or two BC_2F_1 plants. Each line was grown in a separate plot measuring 1.5 m×0.75 m. Fertilizer was broadcast at the rate of 60 kg ha^{-1} N and 70 kg ha^{-1} P before planting. The plots were sprayed with the fungicide Tilt (propiconazole) twice during the vegetative growth phase as a preventive disease control measure against foliar diseases such as leaf rust and powdery mildew.

Trait evaluation

Days to heading (HD) were noted when 50% of spikes had completely emerged from the boot. Plant height (PHT) was calculated as distance from the soil level to the tips of a random fistful of spikes, averaged across three measurements per plot. Kernels from a sample of ten spikes from each plot were threshed, counted and weighed to estimate number of kernels per spike (KER) and kernel weight in milligrams (KW). Tiller number per square meter (TN) was calculated as spike number of one square meter from each plot. These tillers were oven-dried for 1 week at 32°C and weighed for the estimation of aboveground biomass (BM) in ton/ha. Grain yield (YLD) was measured from each plot and harvest index (HAI) was computed as YLD/BM. Test weights (TW) and moisture content were measured with a grain analysis computer (Dickey John GAC 2000).

Table 1 Development of the backcross population from the cross TA4152-4 × Karl 92

	BC_1 plant ID	BC_2F_1 family ID	Number of BC_2F_1 plants	Number of $BC_2F_{2:4}$ lines	Numbers of $BC_2F_{2:4}$ lines, by BC_2F_1 plant
	1	1	9	56	8, 7, 3, 6, 6, 12, 6, 1, 7
	1	2	7	57	23, 8, 5, 5, 7, 1, 8
	1	3	5	19	5, 2, 2, 1, 9
	1	4	4	6	1, 1, 2, 1, 1
	1	5	3	21	10, 9, 2
	1	6	2	6	4, 2
	2	7	2	6	1, 5
	3	8	2	19	16, 3
Total selected	3	8	35	190	
Total developed	3	15	120	1,200	

Grain protein content (PROT) and kernel hardness (HARD) were estimated from a 13 g sample by near-infrared reflectance using a Percon Inframatic 8620. At Manhattan, where incidence of wheat soilborne mosaic virus (WSBMV) was high, each line was scored for severity on a scale of one (highly resistant) to four (highly susceptible).

Analysis of field data

Analysis of variance (ANOVA) was performed to estimate genotype and location effects for all the traits using SAS (SAS Institute, 1999). Pearson's correlation coefficients were calculated for each trait and location combination.

Genotypic (line within family) and error variances and covariances within each environment were estimated by the method of moments using the MANOVA option of SAS PROC GLM (SAS Institute, 1999) for each pair of traits. Genotypic correlations were estimated based on the variance and covariance estimators and their approximate standard errors were estimated using the delta method (Mode and Robinson 1959).

Marker analysis

Genomic DNA was isolated from fresh young leaves of the parents according to the protocol of Riede and Anderson (1996). A polymorphism survey of the parents was carried out with 666 SSR markers comprising 174 CFA and CFD SSRs (Sourdille et al. 2003), 246 GWM SSRs with dinucleotide repeats (Röder et al. 1998a, b), 65 GDM SSRs (Pestsova et al. 2000), 141 SSRs with prefix BARC (Song et al. 2000) and 40 SSRs derived from the wheat EST database designated with the prefix KSM (Singh et al. 2000). Polymerase chain reactions were performed as described by Röder et al. (1998b). Amplified products were separated on 6% non-denaturing polyacrylamide gels in TRIS borate buffer or 2.3% SFR agarose gels (Genemate, ISC Bioexpress). Gels were stained with ethidium bromide and visualized under UV light.

Total genomic DNA was extracted from at least 20 plants of each $BC_2F_{2:4}$ line and 154 polymorphic microsatellite markers were used to genotype the population. Due to the loss of one of the lines only 189 of the 190 lines were used for genotyping. Altar 84 and WX 193 were included in the polymorphism survey to aid in genomic assignment of 35 fragments from 18 markers. The linkage map used for analysis was based on the marker order and relative distances in the International Triticeae Mapping Initiative (ITMI) population (Somers et al. 2004).

Trait analysis

QTL analysis was performed with the QGene 3.07a software program (Nelson 1997). Chi-square tests were employed to estimate segregation distortion in the population. Location-wise data were analyzed separately owing to weak correlations between locations. The association between phenotype and marker genotype was evaluated by single-marker regression and positions of detected QTLs were determined approximately using simple interval mapping (Haley and Knott 1992) based on a BC_2S_1 model. The proportion of phenotypic variance explained by a QTL was estimated as the coefficient of determination (R^2) of the closest marker. A QTL was declared significant if the regression F statistic reached or exceeded the value corresponding to an experiment-wise permutation threshold of $P < 0.05$ for that trait–environment combination. This threshold was calculated as the ninety-fifth percentile of the distribution of maximum F statistics recorded for each of 5,000 regressions of reshuffled trait data on all 151 markers.

Environmental (E) and QTL × E interaction effects were estimated as described by Pillen et al. (2003), using SAS PROC GLM with a model incorporating marker, environment and marker-by-environment effect for markers declared significantly associated with a trait. For this purpose, incomplete values of dominant markers and missing genotypes were replaced with their conditional expectations calculated according to Jiang and Zeng (1995). Both additive and dominance effects were estimated. Using unconditional expectations (from expected genotype segregation) and not partitioning genetic effects gave almost the same results.

Results

Microsatellite polymorphism and marker segregation

Of the 666 microsatellite markers tested on the parents, 154 (23%) were polymorphic and were used to genotype the BC_2F_2-derived population. Three markers on chromosomes 1D, 2A and 6D did not segregate. The D genome had the most polymorphic markers (46%), followed by the B (31%) and A genomes (23%). Marker coverage ranged from 17 on chromosome 2D (Fig. 1) to 3 on chromosomes 1A and 7A, with large gaps (> 50 cM) on chromosomes 3B, 4D, 5D, 6B, 7A and 7D.

The population had on average 4% of lines homozygous for the donor allele and 5.25% of the lines segregating for the donor allele, which is somewhat lesser than the expected values of 12.5 and 6.25% in an unselected BC_2F_2-derived population. Of the 151 markers scored, 54 (35%) deviated ($P < 0.001$) from the expected ratio. For five markers on four different chromosomes (2B, 6B, 7B and 7D), more lines were homozygous for the synthetic parent allele than expected. Otherwise, segregation was skewed towards the recurrent-parent allele, especially on chromosomes 2D (7 markers), 3D (9 markers) and 6D (4 markers).

Trait variation and correlations

The means of the BC_2F_2-derived population were higher than the recurrent-parent (Karl 92) mean for TN, BM, TW, YLD, PHT and HD and lower for KER, KW and HAI (Table 2). Since the non-winter-hardy synthetic hexaploid parent could not be evaluated in the same environment, it was not possible to assess transgressive segregation in the population. Variation among genotypes within each block was observed ($P < 0.001$) for all traits except BM (Table 3). Location effects were seen ($P < 0.001$) for all variables except KW. Although location × family was significant for all variables except KW, significant location × genotype interactions were observed only for PHT and TW. However, for both traits these interaction mean squares were exceeded by the mean squares for entries within families (genotypic effect).

Correlation coefficients between traits were calculated separately for each location (Table 4). The strongest correlation was detected between TN and BM at both locations. Heading date was positively correlated ($P < 0.001$) with PHT and HARD and negatively with KW and YLD. YLD was positively correlated with KW, BM and TW and negatively with HAI. PHT was positively correlated with KER and BM and negatively with HAI. The severity of WSBMV was correlated positively with HD and HAI and negatively with PHT and BM.

The few genotypic correlations whose estimates exceeded their standard error by a factor of at least 2 ($P \leq \sim 0.05$) are also shown in Table 4. For HD and PHT, the value was similar to the phenotypic correlation value in both locations. For other traits where both kinds of correlations were significant in the same environment, signs were the same though values were often quite different.

QTL detection

Putative QTLs for seven traits are summarized in Table 5 and their map positions are shown in Fig. 1.

Days to heading Two QTLs associated with HD were identified. Alleles from the synthetic hexaploid parent located on chromosome arms 2DS (QHd.ksu-2D) and 3DS (QHd.ksu-3D) increased HD by 1.2% (1.5 days) at Hutchinson and 0.8% (1 day) at Manhattan, respectively ($R^2 = 0.09$–0.12).

Kernels per spike One QTL was found on chromosome arm 3DS (QKer.ksu-3D, $R^2 = 0.12$), with the synthetic allele increasing KER by 11% at Manhattan.

Tiller number One putative QTL ($R^2 = 0.06$) was detected on chromosome arm 3BL (QTn.ksu-3B) with the

Table 2 Means and ranges of the BC_2F_2-derived lines and the recurrent parent

	LSD	Manhattan			Hutchinson		
		Lines	Range	Karl 92	Lines	Range	Karl 92
HD (Julian days)	1.6	128	123–134	127	127	124–131	127
PHT (cm)	4.1	95	83–107	92	89	73–102	83
KER	4.3	25.4	18–39	26.9	26.6	20–35	28.6
KW (mg)	3.4	34.3	27.6–42.3	35.7	34.4	29.2–40.2	35.1
TN/m^2	26	122	63–186	114	113	70–156	102
BM (ton/ha)	4.1	17.1	11.1–24.7	16.8	16.4	10.3–22.3	16.3
HAI	0.06	0.32	0.20–0.43	0.36	0.36	0.26–0.46	0.40
TW (Kg/m^3)	18	748	646–771	746	764	704–811	760
YLD (Kg/ha)	755	5924	3209–7743	5897	5670	3672–7121	5144
PROT	2.87	19.30	17.07–25.10	19.10	21.72	17.16–26.61	21.26
HARD	9.18	67.64	25.37–88.17	71.32	56.37	17.71–83.35	58.83

HD days to heading; *PHT* plant height; *KER* kernels per spike; *KW* kernel weight; *TN* Tiller number; *BM* biomass; *HAI* harvest index; *TW* test weight; *YLD* grain yield; *PROT* grain protein content; *HARD* kernel hardness

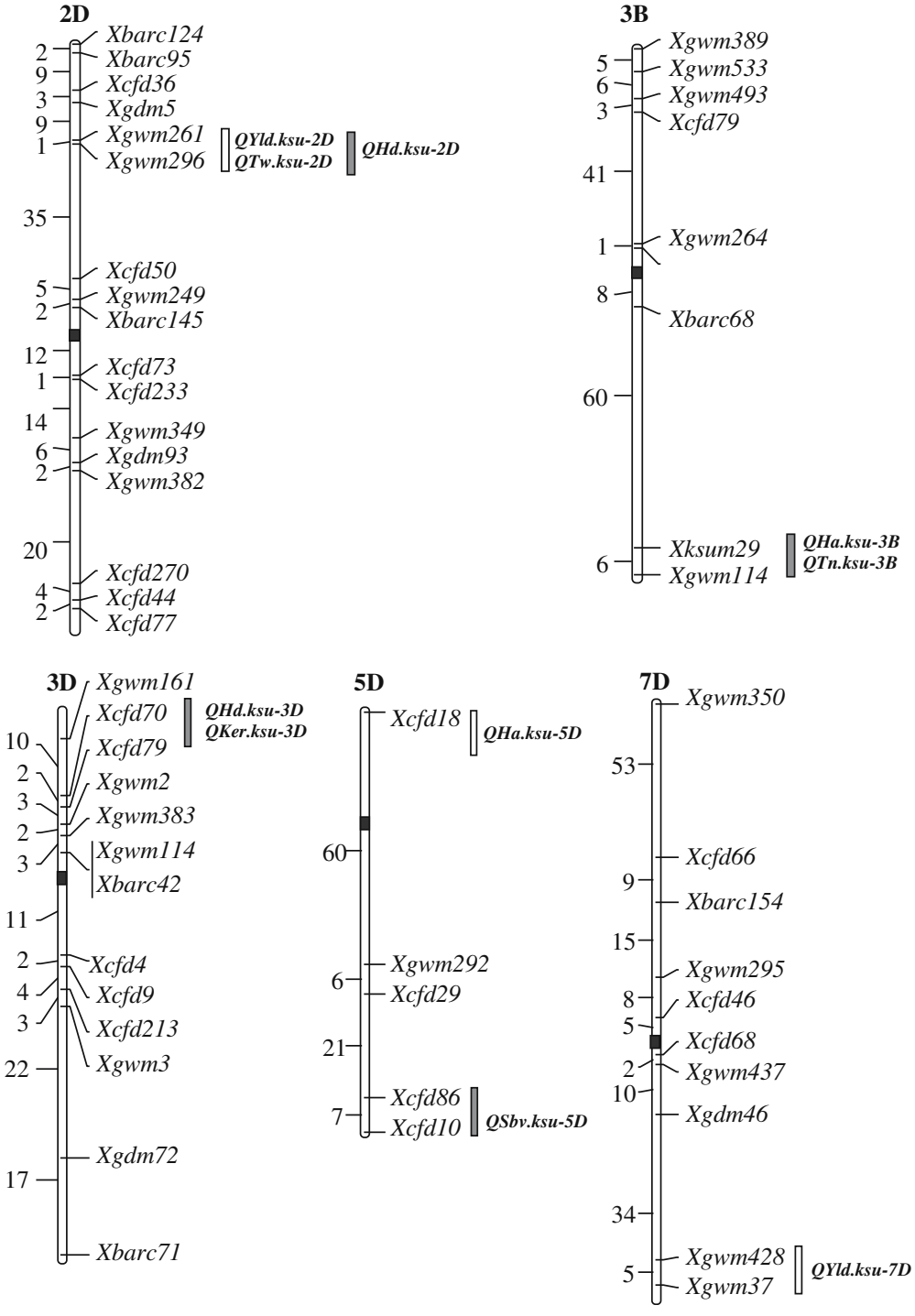

Fig. 1 Linkage map of microsatellite markers used for BC2F2 QTL analysis. Marker order and relative distances are based on the ITMI population (Somers et al. 2004). Abbreviations for traits: *QHd.ksu* days to heading; *QKer.ksu* kernels per spike; *QTn.ksu* Tiller number per m^2; *QTw.ksu* grain volume weight; *QYld.ksu* grain yield; *QHa.ksu* kernel hardness; *QSbv.ksu* soil borne mosaic virus. *Gray vertical bars* indicate QTLs from TA4152-4. *Open bars* indicate QTLs from Karl 92

synthetic allele increasing by 7.3% TN at Hutchinson.

Test weight A single putative QTL (QTw.ksu-2D, $R^2 = 0.32$) was found for TW at Manhattan, with 2.3% increase in TW by the same 2DS Karl 92 allele associated with decreased time to heading at Hutchinson.

Grain yield At Manhattan significant association between YLD and the same 2DS marker allele from Karl 92 that increased test weight was identified. This QTL, designated QYld.ksu-2D, accounted for about 13% of yield variation. A QTL on chromosome arm 7DL, QYld.ksu-7D ($R^2 = 0.09$), increased yield at

Table 3 Mean squares for yield and yield components for BC_2F_2-derived population tested at Manhattan and Hutchinson in 2002–2003

Source of variation	*df*	Mean squares										
		HD	PHT	KER	KW	TN	BM	HAI	TW	YLD	PROT	HARD
Loc	1	102.47***	10710.22***	300.46***	0.53	20660.97***	88.76**	0.270***	67348.22***	3335793.6***	1469.17***	40306.99***
Rep(loc)	4	20.28***	106.15***	11.37	165.67***	4833.66***	311.78***	0.160***	1518.54***	14117077.6***	0.49	235.20
family	19	47.79***	536.72***	166.74**	42.13**	1067.16	15.02	0.012	3238.41**	5369345.2**	21.27*	8669.50***
Entry(fam)	192	5.75***	67.72***	18.70**	13.47***	641.26**	8.63	0.004**	402.26***	1387585.2***	5.84**	538.45***
Fam*loc	19	5.81***	33.77***	46.69***	9.97	926.16**	22.11**	0.009***	807.76***	1349839.4**	7.31***	332.37***
Loc*entry(fam)	192	1.80	12.76*	13.42	8.29	406.42	9.91	0.002	244.15**	754057.4	4.12***	67.57
Error	800	1.48	10.03	12.20	8.01	406.79	9.29	0.002	173.83	686714	2.70	78.44
R^2		0.70	0.83	0.53	0.48	0.53	0.48	0.64	0.67	0.53	0.65	0.84
CV		0.96	3.17	13.35	8.22	17.51	18.35	13.70	1.75	14.33	8.05	14.18

HD days to heading; *PHT* plant height; *KER* kernels per spike; *KW* kernel weight; *TN* Tiller number; *BM* biomass; *HAI* harvest index; *TW* test weight; *YLD* grain yield; *PROT* grain protein content; *HARD* kernel hardness

*, **, *** Significant at $P < 0.05$, $P < 0.01$, $P < 0.001$, respectively

Hutchinson by about 8% with the increased effect from the recurrent parent.

Hardness Two putative QTLs were identified for HA at both locations. The Karl 92 allele increased hardness by 19% at the major locus on chromosome arm 5DS ($R^2 = 0.30$), and decreased it by the same amount at the other QTL on chromosome arm 3BL, QHa.ksu-3B ($R^2 = 0.17$).

Wheat soilborne mosaic virus A major QTL was found on chromosome arm 5DL QSbv.ksu-5D ($R^2 = 0.38$), with Karl 92 alleles increasing resistance.

Discussion

Segregation effects of selection

The excess of progeny homozygous for the recurrent-parent allele in our population was expected due to the selection applied against the traits from the synthetic parent. Previous AB-QTL studies have reported similar deviations in wheat (Huang et al. 2003), rice (Moncado et al. 2001) and tomato (Fulton et al. 2000). The wild diploid parent *Ae. tauschii* of the synthetic hexaploid wheat contributed the genes for shattering and tough glumes. Despite this negative selection, many polymorphic D-genome markers segregated in the population.

Location and QTL × E effects

The location effects detected for all traits except KW, which showed no QTLs, may have been aggravated by a high level of wheat soilborne mosaic virus infection and cooler-than-normal temperatures that increased the grain fill duration at Manhattan. The Manhattan location also received more precipitation during the later stages of plant development. In accounting for trait variation using any single QTL-linked marker, QTL × E effects were seen for a few QTLs, but the variation this explained was swamped by location (environmental) variation (Table 5) except for the TW QTL, where both effects were strong.

QTL detection and comparison

The ten putative QTLs identified for seven traits were associated with six distinct markers (Table 2). The synthetic wheat accession TA 4152-4 contributed the desirable allele for two yield-component traits. Three QTLs were expressed only at Hutchinson and five only at Manhattan, while two kernel-hardness QTLs were expressed at both locations (Table 5). No dominance effect was significant.

Table 4 Correlations between traits in the BC_2F_2-derived population for Manhattan and Hutchinson. Upper values are for Manhattan and lower values are for Hutchinson

Trait	HD	PHT	KER	KW	BM	TN	HAI	TW	YLD	PROT	HARD	WSBMV
HD		0.411*						−0.246*			0.190*	
		0.393*	−0.454*								0.325*	
PHT	0.429***											
	0.297***		−0.467*					0.726**	0.941***			
KER	0.351***	0.283***		0.732**								
KW	−0.162*											
	−0.233***		−0.175 *								−0.662*	
BM		0.188**										
		0.225**										
TN				−0.278***	0.549***							
			−0.279***	−0.240***	0.707***							
HAI	−0.292***	−0.247***		0.252***		−0.166*						
	−0.174*	−0.285***		0.146*	−0.144*	−0.224***						
TW			−0.126**						0.380*		0.369*	
		0.194**	0.224**	0.247***								
YLD	−0.344 ***			0.268***	0.154*		−0.436***	0.439***			−0.272*	
	−0.225**			0.219**			−0.323***	0.378***			−0.411*	
PROT				−0.157*		0.188*	−0.261***	0.176*				
				0.028								
HARD	0.314***			−0.185*				0.354***				
	0.330***			−0.278*		0.149*				−0.223*		
WSBMV	0.173**				−0.139*		0.166*	0.147*			0.275***	

In the upper diagonal are genotypic correlations; in the lower, phenotypic correlations
HD days to heading; *PHT* plant height; *KER* kernels per spike; *KW* kernel weight; *TN* Tiller number; *BM* biomass; *HAI* harvest index; *TW* test weight; *YLD* grain yield; *PROT* grain protein content; *HARD* kernel hardness; *WSMV* wheat soilborne mosaic virus
*, **, *** Significant at $P < 0.05$, $P < 0.01$, $P < 0.001$, respectively

One of the major hardness QTLs coincided with the well-known *Ha* (Law et al. 1978; Sourdille et al. 1996; Perretant et al. 2000) and *Gsp* puroindoline genes (Igrejas et al. 2002) on 5DS, while the other QTL on chromosome arm 3BL coincided with none previously reported for the trait. The 5DL region that accounted for 37% of the variation in disease severity is the first report of chromosome location of a gene for resistance to WSBMV. The QTL for Hutchinson heading-date *QHd.ksu-2D* is linked with the same microsatellite locus *Xgwm261*-2DS reported by Sourdille et al. (2000) to be associated with heading-date gene *Ppd-D1*. The QTL on chromosome arm 3DS influencing heading at Manhattan has not previously been reported. The *QKer.ksu-3D*

Table 5 Putative QTLs detected in the backcross population from the cross TA4152-4 × Karl 92

Trait	QTL	Marker	Increased effect[a]	%A[b]	Single-marker analysis		Interval Mapping		Location[e]	E[f]	QTL × E[g]
					LOD[c]	R^2 (%)	LOD[d]	R^2 (%)			
HD	*QHd.ksu-2D*	*Xgwm261*	SH	1.20	3.94	0.170	4.03	0.066	H	***	NS
	QHd.ksu-3D	*Xgwm161*	SH	0.78	5.01	0.116	4.98	0.105	M	***	*
KER	*QKer.ksu-3D*	*Xgwm161*	SH	11.0	5.35	0.123	5.60	0.132	M	***	*
TN	*QTn.ksu-3B*	*Xksum29*	SH	7.26	3.55	0.062	3.03	0.034	H	***	NS
TW	*QTw.ksu-2D*	*Xgwm261*	K	−2.32	15.8	0.323	9.01	0.097	M	***	***
YLD	*QYld.ksu-2D*	*Xgwm261*	K	−12.3	5.82	0.133	6.04	0.122	M	***	*
	QYld.ksu-7D	*Xgwm37*	K	−7.85	3.74	0.088	4.01	0.090	H	**	NS
WSBMV	*QSbv.ksu-5D*	*Xcfd10*	SH	90.0	18.3	0.376	18.97	0.370	M	–	–
HARD	*QHa.ksu-3B*	*Xksum29*	SH	19.4	3.79^H	0.090	4.09	0.095	M, H	***	NS
	QHa.ksu-5D (Ha)	*Xcfd18*	K	−18.7	14.52^M	0.299	14.6	0.299	M, H	***	NS

HD days to heading; *PHT* plant height; *KER* kernels per spike; *TN* Tiller number; *HAI* harvest index; *TW* test weight; *YLD* grain yield; *PROT* grain protein content; *HARD* kernel hardness; *WSMV* wheat soilborne mosaic virus
[a]Parental source of marker allele with higher mean phenotype: *K*, Karl; *SH*, synthetic parent
[b]%A (additive regression coefficient/AA)×100, where AA is the phenotypic mean of the homozygous Karl allele
[c]LOD score from the most significant location by single-marker analysis
[d]LOD score from the same location by interval mapping
[e]M, Manhattan; H, Hutchinson
[f]Environmental effect *P* value: ***$P < 0.0001$; **$P < 0.001$; *$P < 0.05$; NS $P > 0.05$
[g]QTL × environmental effect *P* value; same notation as for E. Neither effect could be estimated for WSBMV, which was evaluated in only one location

locus on 3DS at which the synthetic hexaploid allele increased kernel number is different from previously reported QTLs for this trait (Shah et al. 1999) and the related traits such as ear and peduncle length (Börner et al. 2002). The putative QTL on chromosome arm 3BL for increased tillers per unit area from the synthetic hexaploid parent is different from QTLs on 3BS identified by Huang et al. (2003) derived from the synthetic hexaploid wheat 'W-7984'. Other reported QTLs for tiller number on chromosome 1D (Li et al. 2002), 3A (Shah et al. 1999) and 7B (Kato et al. 1999, 2000) were not detected in our study. *QYld.ksu-2D*, which strongly influenced grain yield at Manhattan, lies in the same region as a QTL reported by Huang et al. (2003) with the favorable allele contributed by the synthetic parent. In our study, using a different accession of *Ae. tauschii*, the increased effect was due to the recurrent-parent allele. The yield QTL *QYld.ksu-7D* on chromosome arm 7DL from Karl 92 has not been reported elsewhere.

Phenotypic correlations and correspondence with QTL results

As expected, grain yield was positively correlated with the yield components such as biomass, kernel weight and test weight. The negative correlation of days to heading with kernel weight and grain yield may represent the effect of heat stress during grain filling in late-flowering genotypes, despite cooler-than-normal temperatures. A negative correlation between heading and grain yield was observed in a synthetic-hexaploid-derived population (del Blanco et al. 2001). Early heading of wheat cultivars in the southern Great Plains is an important mechanism of heat stress escape. Synthetic hexaploids are generally tall and late heading and in our study, a positive correlation between heading time and plant height suggested a genetic correlation between these traits. In this study increased plant height and tillers per unit area were accompanied by greater aboveground biomass but not increased yield. Similar observations were made by del Blanco et al. (2001). WSBMV correlations at Manhattan were consistent with the delayed heading and stunting characteristic of WSBMV-infected lines.

Some trait correlations can be ascribed to pleiotropic QTLs. The 2DS association with both yield and test weight at Manhattan probably represents a single QTL, in view of both the positive phenotypic correlation between these traits and the high overall similarity (data not shown) of their respective marker associations over the entire set. By the same criteria, the Manhattan heading-date QTL on chromosome arm 3DS may be the same one influencing kernels per spike. If so, the synthetic-parent allele at this locus may be of little practical use, first because later heading is undesirable and second because kernel number has low or negative correlation with test weight and yield. The coincidence of the WSBMV resistance QTL *QSbv.ksu-5D* with a minor locus influencing heading date (not reported) is consistent with the phenotypic correlation between these two traits. The weak correlation between tiller number and hardness at Hutchinson may be due to linkage of the minor QTLs for these traits on 3BL. Again, since tiller number was not correlated with yield, it is uncertain whether this putative QTL would be of practical breeding utility.

Despite the large genotypic correlation between plant height and yield at Hutchinson, no QTL pleiotropic for these traits was found. Genotypic correlations estimate the expected correlated response of one trait to selection on another, in our case within-family selection where "family" refers to descendants of one (occasionally two) BC_2 parents. Here these statistics were biased to an unknown degree by the selection applied during line development and were of low precision owing to the small number of families.

Limitations of the experimental design

Effect of design

The aim of AB-QTL analysis is to identify regions from the exotic material that influence yield traits and simultaneously transfer them to breeding material. Despite its record of some success in these aims, some adverse effects follow from the small number of progeny carrying a donor QTL allele. The small sample of recombinations results in low position resolution and spurious linkages suggesting pleiotropy. The high influence of each of these progeny on contrasts between marker-genotype classes, and the unstable degree of phenotypic variation in the smaller class, inflate the sampling error in the statistic, as reflected in permutation tests, such that only the largest QTLs are reliably detected. The two environments used in our study proved sufficiently different that the only QTLs detected in both were for the highly heritable trait kernel hardness, one for which new alleles are not sought.

The common use of single-marker analysis in AB-QTL studies probably stems from the use of non-standard mating designs not accommodated by interval-mapping software. In any case, since the genotype-frequency models used in interval-mapping methods do not take selection into account, and the variance of QTL location and effect estimates is compounded by the additional rounds of backcrossing with steady loss of observable recombination, it is questionable whether interval mapping offers any advantage over single-marker tests.

Missing QTLs

Altar 84, the tetraploid parent of the synthetic, is a widely grown, high-yielding cultivar in Mexico. In our study since 23% of the lines out yielded the recurrent parent significantly, we expected to detect several yield QTLs with positive effect derived from the synthetic

parent. One reason that we did not may be the uneven genome coverage obtained from the marker set. Examination of the breeding records also revealed that of the 189 advanced lines, 164 (87%) descended from a single BC_1 plant. Even though all but three of the SSR markers polymorphic in the parents still segregated both parental alleles in the advanced lines, this result of selection may have biased the sample of QTL-informative recombination events. Fulton et al. (1997, 2000) derived the mapping population from five and eight BC_1 parents, respectively, while in other studies (Bernacchi et al. 1998; Huang et al. 2003, 2004; Septiningsih et al. 2003; Moncada et al. 2001) the population was based on from 21 to 80 BC_1 parents.

Significance levels

In most advanced backcross QTL studies, relatively low and arbitrarily defined LOD and R^2 thresholds have been used to identify all possible QTLs that might influence the trait (cf. Huang et al. 2003). We chose to limit Type I error and focus on QTL candidates distinguishable from noise by a robust test, since only these would merit attention in the practical breeding programs for which the AB-QTL scheme was devised. Accordingly we rejected many QTL candidates of a type that other experimenters have reported on the strength of P values. It may be that meta-analysis (Goffinet and Gerber 2000) would extract more evidence from the combined studies, provided that their data become available in a public database for such analyses. Our data will be submitted to the GrainGenes database.

Conclusion

This study identified genomic regions in the synthetic parent that increase two yield components and kernel hardness in wheat and also revealed a new major virus-resistance gene in the cultivated parent. The low harvest of novel beneficial alleles from this cross suggests that an advanced-backcross QTL design, especially in a small sample of environments, can achieve useful QTL detection power only when applied to traits of high heritability and low genotype-by-environment interaction, when marker coverage is adequate (especially important in a large genome such as wheat's genome), and when selection in against unwanted wild characters has not been rigorous enough to bias the sample of recombination events informative about QTLs.

In practice, rigorous selection is needed in order to recover adapted lines from crosses between synthetic hexaploids and hard red winter wheat cultivars. It may be more practical in winter wheat to utilize synthetic hexaploids developed from more winter-hardy, facultative-type durum wheats. There are also a large number of elite spring wheat lines developed at CIMMYT from crosses with synthetic hexaploid wheats that may be a more practical source of new genes for winter wheat breeding programs since many of the undesirable traits of the synthetic have already been selected against.

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References

Bernacchi D, Beck-Bunn T, Eshed Y, Lopez J, Petiard V, Uhlig J, Zamir D, Tanksley S (1998) Advanced backcross QTL analysis in tomato. I. Identification of QTLs for traits of agronomic importance from *Lycopersicon hirsutum*. Theor Appl Genet 97:381–397

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

Cox TS (1998) Deepening the wheat gene pool. J Crop Prod Recent Advances 1:1–25

Cox TS, Sears RG, Bequette RK, Martin TJ (1995) Germplasm enhancement in winter wheat × *Triticum tauschii* backcross populations. Crop Sci 35:913–919

del Blanco IA, Rajaram S, Kronstad WE, Reynolds MP (2000) Physiological performance of synthetic hexaploid wheat-derived populations. Crop Sci 40:1257–1263

del Blanco IA, Rajaram S, Kronstad WE (2001) Agronomic potential of synthetic hexaploid wheat-derived populations. Crop Sci 41:670–676

Fritz AK, Cox TS, Gill BS, Sears RG (1995) Marker based analysis of quantitative traits in winter wheat × *Triticum tauschii* populations. Crop Sci 35: 1695–1699

Fulton TM, Beck-Bunn T, Emmatty D, Eshed Y, Lopez J, Petiard V, Uhlig J, Zamir D, Tanksley SD (1997) QTL analysis of an advanced backcross of *Lycopersicon peruvianum* to the cultivated tomato and comparisons with QTLs found in other wild species. Theor Appl Genet 95:881–894

Fulton TM, Grandillo S, Beck-Bunn T, Fridman E, Frampton A, Lopez J, Petiard V, Uhlig J, Zamir D, Tanksley SD (2000) Advanced backcross QTL analysis of a *Lycopersicon esculentum* × *Lycopersicon parviflorum* cross. Theor Appl Genet 100:1025–1042

Goffinet B, Gerber S (2000) Quantitative trait loci: a meta-analysis. Genetics 155:463–473

Haley CS, Knott SA. (1992) A simple regression method for mapping quantitative trait loci in line crosses using flanking markers. Heredity 69:315–324

Ho JC, McCouch SR, Smith ME (2002) Improvement of hybrid yield by advanced backcross QTL analysis in elite maize. Theor Appl Genet 105:440–448

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 aestivumL.*). Theor Appl Genet 109:933–943

Igrejas G, Leroy P, Charmet G, Gaborit T, Marion D, Branlard G (2002) Mapping QTLs for grain hardness and puroindoline content in wheat (*Triticum aestivum L.*). Theor Appl Genet 106:19–27

Jiang C, Zeng ZB (1995) Multiple trait analysis of genetic mapping for quantitative trait loci. Genetics 140:1111–1127

Joshi CP, Nyguen HT (1993) RAPD (random amplified polymorphic DNA) analysis based intervarietal genetic relationships among hexaploid wheats. Plant Sci 93:95–103

Kam-Morgan LNW, Muthukrishnan S, Gill BS (1989) DNA restriction fragment length polymorphisms: a strategy for genetic mapping of the D genome of wheat. Genome 32:724–732

Kato K, Miura H, Sawada S (1999) QTL mapping of genes controlling ear emergence time and plant height on chromosome 5A of wheat. Theor Appl Genet 98:472–477

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

Kihara H (1944) Discovery of the DD-analyser, one of the ancestors of vulgare wheats. Agric Hort (Tokyo) 19:889–890

Ladizinsky G (1985) Founder effect in crop–plant evolution. Eco Bot 39:191–199

Law CN, Snape JW, Worland AJ (1978) The genetic relationship between height and yield in wheat. Heredity 40:133–151

Li WL, Nelson JC, Chu CY, Shi LH, Huang SH, Liu DJ (2002) Chromosomal locations and genetic relationships of tiller and spike characters in wheat. Euphytica 125:357–365

Mode CJ, Robinson HF (1959) Pleiotropism and the genetic variance and covariance. Biometrics 15:518–537

Moncada P, Martínez CP, Borrero J, Chatel M, Gauch H Jr, Guimaraes E, Tohme J, McCouch SR (2001) Quantitative trait loci for yield and yield components in an *Oryza sativa* × *Oryza rufipogon* BC2F2 population evaluated in an upland environment. Theor Appl Genet 102:41–52

McFadden ES, Sears ER (1946) The origin of *Triticum spelta* and its free-threshing hexaploid relatives. J Hered 37:81–89

Mujeeb-Kazi A (2000) Current status of D genome based synthetic hexaploid wheats and the characterization of an elite subset. Ann Wheat Newsl 46:76–79

Mujeeb-Kazi A, Fuentes-Davila G, Villareal RL, Cortes A, Rosas V, Delgado R (2001) Registration of 10 synthetic hexaploid wheat and 6 bread wheat germplasm resistant to Karnal bunt. Crop Sci. 41:274

Nelson JC (1997) QGene: software for marker-based genomic analysis and breeding. Mol Breed 3:239–245

Perretant MR, Cadalen T, Charmet G, Sourdille P, Nicolas P, Boeuf C, Tixier MH, Branlard G, Bernard S, Bernard M (2000) QTL analysis of bread-making quality in wheat using a doubled haploid population. Theor Appl Genet 100: 1167–1175

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

Plaschke J, Ganal MW, Röder MS (1995) Detection of genetic diversity in closely related bread wheat using microsatellite markers. Theor Appl Genet 91:1001–1007

Pillen K, Zacharias A, Leon J (2003) Advanced backcross QTL analysis in barley (*Hordeum vulgare L.*). Theor Appl Genet 107:340–352

Rao GU, Ben Chaim A, Borovsky, Paran I (2003) Mapping of yield-related QTLs in pepper in an interspecific cross of *Capsicum annum* and *C. frutescens*. Theor Appl Genet 106:1457–1466

Riede CR, Anderson JA (1996) Linkage of RFLP markers to aluminum tolerance gene in wheat. Crop Sci 36:905–909

Roder MS, Korzun V, Gill BS, Ganal MW (1998a) The physical mapping of microsatellite markers in wheat. Genome 41:278–283

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

SAS Institute Inc (1999) The SAS system for Windows, release 8.00. SAS Institute, Cary, NC

Sears RG, Martin TJ, Cox TS, Chung OK, Curran SP, Heer WF, Witt MD (1997) Registration of Karl 92 wheat. Crop Sci 37:628

Septiningsih EM, Prasetiyono J, Lubis E, Tai TH, Tjubaryat T, Moeljopawiro S, McCouch SR (2003) Identification of quantitative trait loci for yield and yield components in advanced backcross population derived from the *Oryza sativa* variety IR64 and the wild relative *O. rufipogan*. Theor Appl Genet 107:1419–1432

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

Siedler H, Messmer MM, Schachermayr GM, Winzeler H, Winzeler M, Keller B (1994) Genetic diversity in European wheat and spelt breeding material based on RFLP data. Theor Appl Genet 88: 994–1003

Singh S, Li W, Song QJ, Cregan P, Brown-Guedira GL, Gill BS (2000) Development and physical mapping of microsatellite markers in wheat. In: Proceedings National Fusarium Head Blight Forum. Cincinnati, KY, pp 52–53

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, Fickus EW, Cregan PB (2000) Construction of genomic library enriched with microsatellite sequences. Proceedings National Fusarium Head Blight Forum. Cincinnati, KY, p. 50–51

Sourdille P, Perretant MR, Charmet G, Leroy P, Gautier MF, Jourdier P, Nelson JC, Sorrels ME, Bernard M (1996) Linkage between RFLP markers and genes affecting kernel hardness in wheat. Theor Appl Genet 93:580–586

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, Guyomarc'h H, Snape JW, Perretant MR, Charmet G, Boeuf C, Bernard S, Bernard M (2003) An update of the Courtot × Chinese spring intervarietal molecular marker linkage map for the QTL detection of agronomic traits in wheat. Theor Appl Genet 106:530–538

Tanksley SD, Nelson JC (1996) Advanced backcross QTL analysis: a method for the simultaneous discovery and transfer of valuable QTLs from unadapted germplasm into elite breeding lines. Theor Appl Genet 92:191–203

Tanksley SD, Grandillo S, Fulton TM, Zamir D, Eshed T, Petiard V, Lopez J, Beck-Bunn T (1996) Advanced backcross QTL analysis in a cross between an elite processing line of tomato and its wild relative *L. pimpinellifolium*. Theor Appl Genet 92:213–224

Villareal RL, Sayre K, Bañuelos O, Mujeeb-Kazi A (2001) Registration of four synthetic hexaploid wheat (*Triticum turgidum*/*Aegilops tauschii*) germplasm lines tolerant to waterlogging. Crop Sci. 41:274

Xiao JH, Li JM, Grandillo S, Ahn SN, Yuan LP, Tanksley SD, McCouch SR (1998) Identification of trait-improving quantitative trait loci alleles from a wild rice relative, *Oryza rufipogon*. Genetics 150:899–909