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ORIGINAL PAPER

Molecular mapping of QTLs for Karnal bunt resistance in two recombinant inbred populations of bread wheat

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Abstract Karnal bunt (KB) of wheat, caused by the fungus *Tilletia indica*, is a challenge to the grain industry, owing not to direct yield loss but to quarantine regulations that may restrict international movement of affected grain. Several different sources of resistance to KB have been reported. Understanding the genetics of resistance will facilitate the introgression of resistance into new wheat cultivars. The objectives of this study were to identify quantitative trait loci (QTLs) associated with KB resistance and to identify DNA markers in two recombinant inbred line populations derived from crosses of the susceptible cultivar WH542 with resistant lines HD29 and W485. Populations were evaluated for resistance against the KB pathogen for 3 years at Punjab Agricultural University, Ludhiana, India. Two new QTLs (*Qkb.ksu-5BL.1* and *Qkb.ksu-6BS.1*) with resistance alleles from HD29 were identified and mapped in the intervals *Xgdm116–Xwmc235* on chromosome 5B (deletion bin 5BL9-0.76-0.79) and *Xwmc105–Xgwm88* on chromosome 6B (C-6BS5-0.76). They explained up to 19 and 13% of phenotypic variance, respectively. Another QTL (*Qkb.ksu-4BL.1*) with a resistance allele from W485 mapped in the interval *Xgwm6–Xwmc349* on chromosome 4B (4BL5-0.86-1.00) and explained up to 15% of phenotypic variance. *Qkb.ksu-6BS.1* showed pairwise interactions with loci on chromosomes 3B and 6A. Markers suitable for marker-assisted selection are available for all three QTLs.

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Introduction

Karnal bunt (KB) disease, caused by the fungus *Tilletia indica* Mitra (syn. *Neovossia indica*), was first reported in 1931 from wheat grain samples collected near Karnal, Haryana, India (Mitra 1931). Since then, the disease has been of frequent occurrence in northern India and has been reported in parts of several countries including Afghanistan, Iran, Iraq, Mexico, Nepal, Pakistan, South Africa, and the USA (Bonde et al. 1997; Rush et al. 2005). The fungus attacks the developing kernels where it produces a sorus containing black teliospores. KB is seed- and soil-borne and also has an airborne sporidial stage (Carris et al. 2006; Garrett and Bowden 2002).

Karnal bunt is considered a minor disease because disease incidence is typically low and yield losses are negligible. However, the potential impact of KB on the grain industry remains high because of quarantines against the import of infected grain in many countries. Indirect losses incurred by exporting countries may include the cost of regulatory compliance and enforcement, cost of research, and decreased market access (Rush et al. 2005; Vocke et al. 2002). Direct and indirect losses caused by KB in northwestern Mexico were estimated at US $7 million per year (Brennan et al. 1990), while indirect losses in 2001–2002 in

four counties in northern Texas exceeded US $25 million (Rush et al. 2005).

Development of resistant cultivars is the best option for KB management. Genetic variation for KB resistance is found in cultivated wheat as well as its wild relatives (Multani et al. 1988; Villareal et al. 1995, 1996). The main sources of resistance to KB identified to date are of Indian, Chinese, and Brazilian origin (Fuentes-Dávila and Rajaram 1994). In India, several thousand wheat lines were screened for KB resistance and lines HD29 and W485 were found to be consistently resistant over locations and years (Aujla et al. 1992; Sharma et al. 2004).

Genetic studies of KB resistance in wheat have identified from one to nine resistance loci in a set of donors. Different resistance genes in six resistant lines were designated as *Kb1*, *Kb2*, *Kb3*, *Kb4*, *Kb5*, and *Kb6*, but their chromosome locations were not determined (Fuentes-Dávila et al. 1995). Line HD29 was reported to carry three genes for resistance to KB (Harjit-Singh et al. 1999). A recent study reported two resistance genes each in HD29, W485, and a line derived from the cross Aldan 'S'/IAS58, and three resistance genes in a line derived from the cross H567.71/3*Parula (Sharma et al. 2005). Analysis of resistant/resistant crosses showed that the genes in the four stocks were different, indicating as many as nine loci controlling KB resistance in the parents (Sharma et al. 2005). Other studies indicated additive and partially dominant genes dispersed at different loci of wheat (Fuentes-Dávila et al. 1995; Morgunov et al. 1994; Singh et al. 1995a, b; Villareal et al. 1995). RFLP markers on chromosome arms 3BS and 5AL were associated with KB resistance originating in the durum parent of a synthetic hexaploid wheat (Nelson et al. 1998). In another study using PCR-based DNA markers to dissect KB resistance in a recombinant inbred line (RIL) population from the cross HD29/WL711, chromosome 4B was reported to carry a quantitative trait locus (QTL) for KB resistance, which was designated *Qkb.ksu-4BL.1* (Sukhwinder-Singh et al. 2003).

Conventional KB screening is time-consuming and labor-intensive, because inoculations are performed by manual injection of sporidia into the boot and evaluations are done by counting infected and non-infected kernels. Because symptoms are scored on mature grain, crosses with resistant selections often must be delayed to the subsequent growing cycle. Disease expression is highly influenced by environmental conditions and escapes are common. Field inoculations are prohibited by quarantines in many areas.

In view of these difficulties in screening for KB resistance, marker-assisted selection (MAS) would be a useful tool for efficient introgression of resistance into elite breeding material. The objectives of the present study were (1) to identify chromosomal regions associated with KB resistance in two spring bread wheat RIL populations and (2) to develop markers suitable for MAS.

Materials and methods

Plant materials

The two RIL populations used in this study were developed by single-seed descent of individual F_2 plants to F_6 followed by further generations of advance using bulked samples. Population 1 (P1) comprised 109 RILs derived from the cross WH542/HD29 and population 2 (P2) comprised 115 RILs from the cross WH542/W485. All parents are Indian spring wheats. HD29 and W485 are resistant but not immune to KB. WH542 is a highly susceptible cultivar derived from the widely adapted CIMMYT breeding line Kauz (Jupateco/Bluejay//Ures).

Karnal bunt inoculation and disease scoring

The RIL populations were screened for KB resistance at the F_8 to F_{10} generations at Punjab Agricultural University (PAU), Ludhiana, India during crop seasons 2001–2002, 2002–2003, and 2003–2004. RILs were grown in plots consisting of two 1-m rows, with 10 cm spacing between plants. Parents were planted similarly in four-row plots. The RILs and parents were grown in a completely randomized design with three replications in each year. The time of sowing was adjusted so that plants reached the boot stage in the month of February, which is conducive for disease development.

The *T. indica* inoculum mixture comprised 12 isolates representing pathogen variation in the northwestern plains of India (Sharma et al. 2004). The inoculum suspension was injected into the boot of randomly selected tillers (Aujla et al. 1982). At maturity, the inoculated heads were harvested and threshed separately, and the percentage of infected grains in each head was recorded. Mean percentage incidence of disease was determined from five inoculated spikes per plot. Analysis of variance was performed using the SAS (SAS Institute; Cary, NC, USA) GLM procedure with RIL and year effects treated as random. Spearman correlation coefficients (r) among years were estimated on the adjusted means of the RILs using the SAS CORR procedure for each population.

Marker genotyping

Leaf tissue was harvested in bulk from 15 to 20 plants of each RIL, the parents, Chinese Spring (CS) wheat, and the CS aneuploid and deletion lines. Tissue was ground in liquid nitrogen and genomic DNA was extracted using the CTAB-DNA method (Saghai-Maroof et al. 1984). Polymorphism between parents was assessed with PCR-based DNA markers including 1,010 SSRs (Pestsova et al. 2001; Röder et al. 1998; Song et al. 2005; Sourdille et al. 2004;

Yu et al. 2004) and 140 EST-STS markers designed from physically mapped and characterized genes on deletion stocks of wheat (Erayman et al. 2004, http://wheat.pw.usda.gov/cgi-bin/westsql/mapimage.cgi). Genomic DNA amplified with SSR primers was separated on 2.3% GenePure HiRes Agarose (ISC Bioexpress, Kaysville, UT, USA) gels or an ABI 3100 Prism Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) using 36-cm capillaries. Genescan and Genotyper analysis software (Applied Biosystems) were used to score the individuals (Oetting et al. 1995). PCR products from EST-STS primers were separated using the mutation detection enhancement (MDE®) matrix (Cambrex, Rockland, ME, USA) (Bassam et al. 1991; Martins-Lopes et al. 2001).

In both populations, chromosome 4B, 5B, and 6B were targeted for high-density marker genotyping when early analysis suggested that these regions carried QTLs, with the consequence that several other chromosomes are represented by few markers. Genetic linkage maps were constructed with MAPMAKER version 2.0 for Macintosh (Lander et al. 1987). Markers within groups were ordered at LOD 3.0.

QTL analysis

Interval QTL analysis was performed with custom-written MATLAB (MathWorks, Natick, MA, USA) programs. For composite interval mapping (CIM), two cofactor markers were selected by forward stepwise regression performed for each year separately. Joint CIM analysis (JA) of all 3 years was performed by multiple-trait mapping (Zeng 1994) with KB scores from years treated as correlated traits sampled from a multivariate normal distribution. For JA, two cofactors were selected independently for each of the years and the four with greatest effect were used. The LOD score for main QTL effect was calculated based on the null hypothesis H_0: $\beta_1 = \beta_2 = \beta_3 = 0$ (where β_t represents the effect estimated in the tth year) and alternative hypothesis H_A: at least one $\beta_t \neq 0$. QTL by year (QTL × Y) interaction was estimated based on the null hypothesis H_0: interaction term $a = 0$ and alternative hypothesis H_A: $a \neq 0$. Empirical LOD thresholds at a significance level of $\alpha = 0.05$ were estimated for CIM and JA from 1,000 permutations (Churchill and Doerge 1994). For JA, permutation analysis shuffled line records for all 3 years together in order to preserve the correlations between years.

Physical mapping of PCR-based markers

Molecular markers showing significant association with KB resistance in this study were placed on the physical (deletion) map of wheat using nullisomic and ditelosomic lines. The CS and CS aneuploid lines used in the study were obtained from the Wheat Genetics Resource Center at Kansas State University. These included: nullisomic–tetrasomic (N5BT5D, N4BT4D, N6BT6D) lines (Sears 1966); ditelosomic lines 4BS (Dt4BS), 5BL (Dt5BL), and 6BS and 6BL (Dt6BS and Dt6BL) (Sears and Sears 1979); and CS deletion lines for the chromosome 4B long arm (4BL-1, FL = 0.71; 4BL-5, FL = 0.86), 5B long arm (5BL-6, FL = 0.29; 5BL-1, FL = 0.55; 5BL-14, FL = 0.75, 5BL-9, FL = 0.76 and 5BL-16, FL = 0.79), and 6B short arm (6BS-2, FL = 1.05; 6BS-5, FL = 0.76) (Endo and Gill 1996). Chromosome deletion breakpoints and deletion bin names are labeled according to Qi et al. (2003).

QTL interaction tests

Regression-based simple interval mapping (Haley and Knott 1992) was used to identify QTLs interacting with the two QTLs on 5B and 6B. The conditional genotype expectation of either primary QTL as a fixed factor was calculated from its flanking-marker genotypes and a one-dimensional scan with a 1-cM step was performed by regression of phenotype on the product of this expectation with that of each putative QTL. An empirical $p = 0.05$ LOD threshold of 2.2 was estimated from 1,000 permutations.

Results

Phenotypic evaluation

Adequate disease pressure was obtained in all 3 years. Resistant parents HD29 and W485, with mean KB scores of 3–4% over years and a range of 0–5%, were clearly differentiated from the susceptible parent, WH542 with mean KB scores of 44–51% in different years (Table 1). The KB scores of RILs ranged from 0 to 100% (Table 1). The distribution of percent KB infection in both RIL populations was continuous and skewed toward lower percent infection in each year (Figs. 1, 2). Percent KB infection among years was significantly ($p \leq 0.0001$) correlated, ($r = 0.50$–0.87 in P1 and 0.70–0.88 in P2). Effects of RILs, years, and RILs × year were significant ($p \leq 0.0001$) (Table 2).

Marker analysis

Of the 1,150 markers screened, 130 were scored in P1 and 150 in P2. Linkage analysis yielded 20 linkage groups in P1 and 24 in P2. There were five markers in P1 and 22 in P2 that could not be placed in linkage groups. The order of markers in linkage groups generally agreed with those in the published maps (Röder et al. 1998; Somers et al. 2004;

Table 1 Mean and range of Karnal bunt infection in two mapping populations and their parents over 3 years

Year[b]	P1[a]		P2[a]		Parents					
	(WH542/HD29)		(WH542/W485)		WH542		HD29		W485	
	Mean[c]	Range[d]	Mean	Range	Mean	Range	Mean	Range	Mean	Range
2002 (F8)	16.3	0–100	13.5	0–82	43.8	25–59	3.1	0–4	3.0	0–4
2003 (F9)	13.1	0–100	17.8	0–75	50.8	32–67	3.5	0–5	3.3	0–5
2004 (F10)	16.9	0–88	14.1	0–71	48.7	34–63	3.4	0–5	3.1	0–5

[a] Recombinant inbred line sets P1 (n = 109) and P2 (n = 115)

[b] Year of inoculation trial and generation of mapping population

[c] Mean percent infected kernels on five inoculated spikes in each of three replications

[d] Range of percent infected kernels on individual inoculated spikes

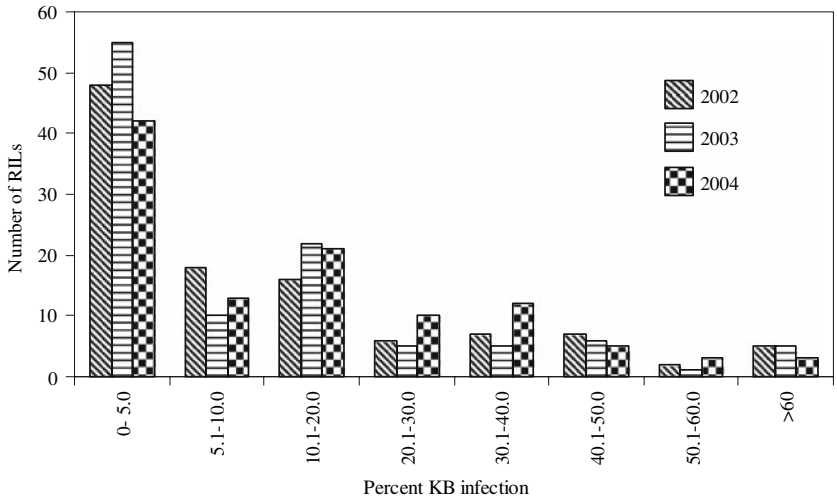

Fig. 1 Distribution of Karnal bunt infection over 3 years in RILs of population P1 developed from the cross WH542/HD29

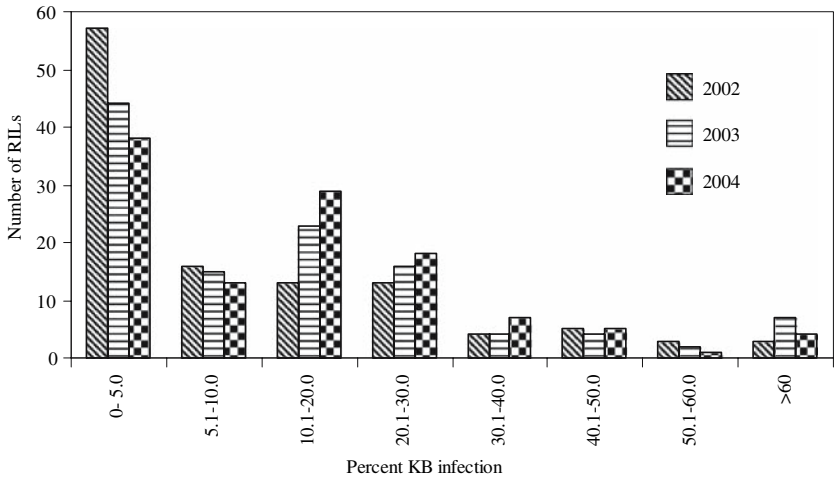

Fig. 2 Distribution of Karnal bunt infection over 3 years in RILs of population P2 developed from the cross WH542/W485

Song et al. 2005; Sourdille et al. 2004). The chromosome coverage in P1 and P2 is given as supplementary figures. Total map coverage was ~1,439 cM in P1 and ~1,040 cM in P2, amounting to 56 and 40% of the 2,560-cM consensus map of Somers et al. (2004). At least half of the markers (75 in P1 and 68 in P2) mapped to the B genome, followed by the A and D genomes. Marker segregation in P1 was highly distorted, with WH542 homozygotes accounting for only 37% of all genotypes. This distortion was spread fairly uniformly over all linkage groups. Segregation in P2 was more balanced, with the WH542 homozygote composing 52% of genotypes.

Table 2 Analysis of variance of Karnal bunt infection in two mapping populations over 3 years

Source	*df*	MS	*F*	*p* value
P1 (WH542/HD29)				
Year	2	1409.0	122.4	<0.0001
RILs	108	2559.5	222.4	<0.0001
Year × RILs	216	411.3	35.7	<0.0001
Error	654	11.5		
P2 (WH542/W485)				
Year	2	198.7	14.6	<0.0001
RILs	114	2155.8	158.5	<0.0001
Year × RILs	228	165.5	12.1	<0.0001
Error	690	13.5		

QTL analysis

In both crosses, QTL disease-reducing effects were associated exclusively with the resistant-parent genotype. QTLs for KB resistance were detected in each year in both populations, and each QTL was well supported by JA across years (Table 3). QTL *Qkb.ksu-6BS.1* in P1 showed an $R^2 = 0.10–0.13$, an additive effect of 8.2–10.8%, and was flanked by markers *Xwmc105* and *Xgwm88* in an 8-cM region (Fig. 3). The other QTL detected in P1, *Qkb.ksu-5BL.1* (Fig. 4), showed an $R^2 = 0.06–0.19$ and an additive effect of 4.2–7.9%. *Qkb.ksu-5BL.1* is in a 5-cM region flanked by SSR markers *Xwmc235* and *Xgdm116*. In P2, QTL *Qkb.ksu-4BL.1* showed an $R^2 = 0.06–0.15$ and an additive effect of 4.2–9.4% (Table 3). It was flanked with markers *Xwmc349* and *Xgwm6* within an interval of 7 cM (Fig. 5).

Two regions on chromosome 3B and 6A showed interaction with *Qkb.ksu-6BS.1* in P1 (Table 4). A 14-cM region on chromosome 3B (*Xgwm493–Xgwm108*) showed a maximum interaction LOD score of 4.7 over years. Another interval (*Xgwm169–Xgwm459*) on chromosome 6A also showed significant interaction with *Qkb.ksu-6BS.1* and an LOD of 4.3. Three of the four recombinant genotypes for these locus pairs appeared more susceptible than even the WH542 parental types (Table 4). No interaction was observed for *Qkb.ksu-5BL.1*. None of the QTLs showed significant QTL × Y interaction in the multiyear analysis.

Assignment of QTLs to chromosome bins

The QTLs detected in the present study were physically localized to chromosome deletion bins by assignment of their flanking markers. Since QTL *Qkb.ksu-5BL.1* was placed between markers *Xwmc235* and *Xgdm116*, primer pairs WMC235 and GDM116 were amplified from genomic DNA of CS, nullitetrasomics, ditelosomics, and deletion stocks for physical mapping. WMC235 amplified two

Table 3 QTLs for Karnal bunt resistance identified in the two RIL populations using composite interval mapping (CIM) and joint analysis (JA)

Map interval (QTL)	Chromosome bin	Analysis method		Year		
				2002	2003	2004
P1 (WH542/HD29)						
Xgdm116 –Xwmc235 (*Qkb.ksu-5BL.1*)	5BL9-0.76-0.79	CIM	LOD[a]	3.5	5.2	1.6
			R^2	0.136	0.194	0.062
			QTL effect[e]	7.4	7.9	4.3
		JA	LOD[b]	5.3		
			R^2	0.136	0.192	0.060
			QTL effect	7.4	7.8	4.2
Xwmc105–Xgwm88 (*Qkb.ksu-6BS.1*)	C-6BS5-0.76	CIM	LOD[a]	3.7	4.1	2.7
			R^2	0.126	0.135	0.098
			QTL effect	10.8	9.6	8.5
		JA	LOD[b]	4.9		
			R^2	0.120	0.119	0.090
			QTL effect	10.38	9.17	8.17
P2 (WH542/W485)						
Xgwm6 –Xwmc349 (*Qkb.ksu-4BL.1*)	4BL5-0.86-1.00	CIM	LOD[c]	3.35	1.98	1.33
			R^2	0.153	0.095	0.057
			QTL effect	9.4	7.0	4.2
		JA	LOD[d]	3.51		
			R^2	0.151	0.103	0.061
			QTL effect	9.3	7.6	4.9

[a] LOD threshold is 3.2 based on 1,000 permutation test

[b] LOD threshold is 3.6; represents the test over all 3 years

[c] LOD threshold is 2.7

[d] LOD threshold is 3.1

[e] QTL additive effect is in units of percent bunted kernels

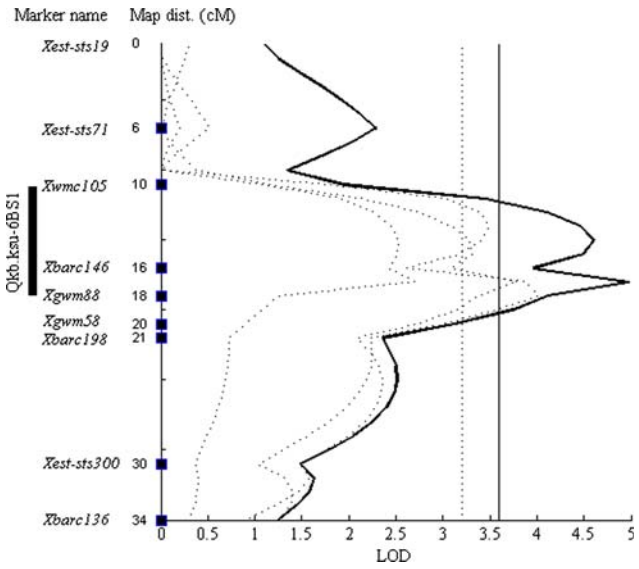

Fig. 3 QTL interval map of *Qkb.ksu-6BS.1* associated with Karnal bunt resistance in recombinant inbred lines (P1) derived from the cross WH542/HD29. The maximum LOD score is indicated on the X-axis. The *dotted* and *solid lines* represent the respective LOD scores for CIM (each of 3 years) and JA

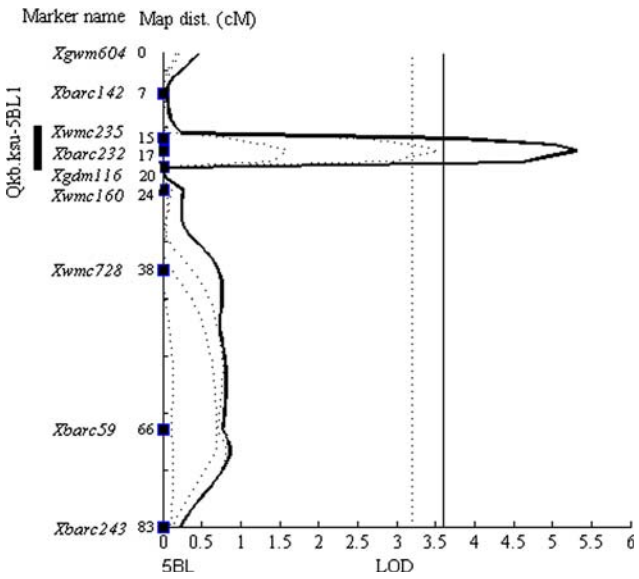

Fig. 4 QTL interval map of *Qkb.ksu-5BL.1* associated with Karnal bunt resistance in recombinant inbred lines (P1) derived from the cross WH542/HD29. The maximum LOD score is indicated on the X-axis. The *dotted* and *solid lines* represent the respective LOD scores for CIM (each of 3 years) and JA

fragments of 250 and 290 bp in CS. The 290 bp fragment was not amplified from DNA of N5BT5D, 5BL-9, 5BL-14, and 5BL-1; however, the corresponding fragment was amplified from DNA of Dt5BL and 5BL-16, confirming the presence of this marker in deletion bin 5BL9-0.76-0.79. The 250-bp fragment amplified in all 5B deletion lines indicated that *Xwmc235* maps to a second chromosome as well.

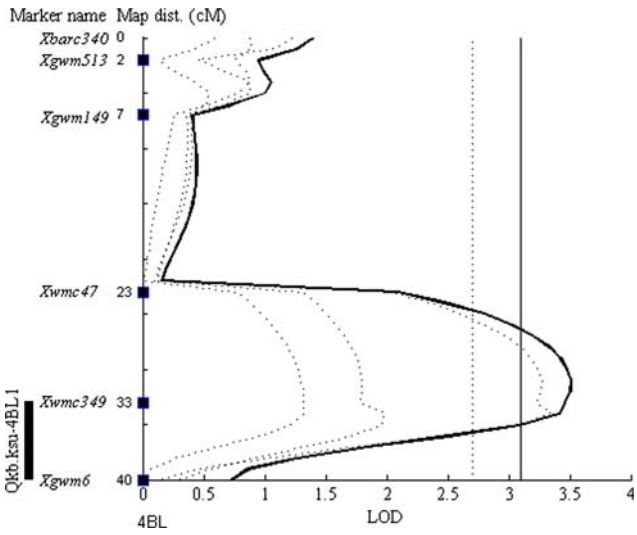

Fig. 5 QTL interval map of *Qkb.ksu-4BL.1* associated with Karnal bunt resistance in recombinant inbred lines (P2) derived from the cross WH542/W485. The maximum LOD score is indicated on the X-axis. The *dotted* and *solid lines* represent the respective LOD scores for CIM and JA

Xgdm116 also mapped to deletion bin 5BL9-0.76-0.79, confirming that *Qkb.ksu-5BL.1* lies in this bin.

The second QTL in P1, *Qkb.ksu-6BS.1*, was similarly localized to bin C-6BS5-0.76. Both of the primer pairs for flanking SSR markers *Xwmc105* and *Xgwm88* amplified two different fragments in CS and WH542. All nulli-tetrasomic lines except N6BT6D showed the target bands, confirming that both markers lie on chromosome 6B. Ditelosomic lines of 6B (Dt6BS and Dt6BL) and deletion lines 6BS-2 and 6BS-5 were screened with both primers and all except Dt6BL showed the fragment, placing both SSRs and their included QTL *Qkb.ksu-6BS.1* in bin C-6BS5-0.76.

The flanking markers *Xgwm6* and *Xwmc349* of QTL *Qkb.ksu-4BL.1* identified in P2 were placed on chromosome 4BL using CS deletion stocks. Fragments amplified from CS but did not amplify from N4BT4D, Dt4BS, 4BL-1, and 4BL-5, placing both markers and the included QTL in the most distal 4BL bin, 4BL5-0.86-1.00.

Discussion

We evaluated two RIL populations derived from crosses of the susceptible cultivar WH542 with resistant lines HD29 and W485 for resistance against the KB pathogen for 3 years at Ludhiana, India. KB scores were continuously distributed (Figs. 1, 2) and resistance was quantitatively inherited as described in earlier reports (Fuentes-Dávila et al. 1995; Nelson et al. 1998; Singh et al. 1995a, b; Sukhwinder-Singh et al. 2003). Two new QTLs, *Qkb.ksu-6BS.1*

Table 4 Interaction effects for Karnal bunt resistance loci in population P1 (WH542/HD29)

Locus A		Locus B		Phenotypic means[a]				Max. LOD[b]
Interval/location	cM	Interval/location	cM	*aabb*	*aaBB*	*AAbb*	*AABB*	
Xwmc105–Xgwm88/6B	8.0	*Xgwm493–Xgwm108*/3B	14.0	12.2 (9)	34.5 (11)	20.8 (18)	5.7 (40)	4.7
(*Qkb.ksu-6BS.1*)		*Xgwm169–Xgwm459*/6A	22.0	18.5 (12)	28.7 (10)	15.6 (9)	11.0 (47)	4.3

[a] Phenotypic mean of percent bunted kernels. *A* and *B* represent alleles from resistant parent HD29 and *a* and *b* the corresponding WH542 alleles. Alleles are those of the first marker in each interval. In parentheses are the sizes of the respective genotype classes

[b] Interaction LOD threshold is 2.2 based on 1,000-permutation test

(Fig. 3) and *Qkb.ksu-5BL.1* (Fig. 4), with resistance alleles from HD29 were identified and mapped on chromosomes 6B and 5B. They explained up to 13 and 19% of phenotypic variance, respectively. A previously reported QTL, *Qkb.ksu-4BL.1* (Fig. 5), with a resistance allele from W485, was identified on chromosome 4B and explained up to 15% of phenotypic variation. All three of these QTLs were statistically significant in multiyear joint CIM analyses.

Interactions of *Qkb.ksu-6BS.1* with regions on chromosomes 3B and 6A were such that the 6BS allele from HD29 conferred resistance only in the presence of HD29 alleles at either of the other loci. This interaction would complicate MAS by requiring transfer of two or three HD29 alleles in any backcrossing scheme. While combinations with resistance alleles at both 6B and 3B showed lowest disease scores, the small genotype class sizes leave unreliable any practical conclusion about three-way interaction.

Qkb.ksu-5BL.1 was the most tightly defined QTL region. Though it represents only 4% of the long arm of 5B, the chromosome bin 5BL9-0.76-0.79 containing QTL *Qkb.ksu-5BL.1* also harbors genes for several other important traits including resistance to Fusarium head blight (Lin et al. 2006), aphids (Miller et al. 2001), and kernel length (Dholakia et al. 2003). It is possible that the 5BL QTL is homologous to the 5AL QTL reported by Nelson et al. (1998).

Qkb.ksu-4BL.1 was originally identified in the cross WL711/HD29, where it accounted for up to 25% of phenotypic variation for KB reaction (Sukhwinder-Singh et al. 2003). A SNP-based marker (*Xgwm538snp*) for this QTL was developed from *Xgwm538*, the SSR marker closest to the originally reported QTL peak (Brooks et al. 2006). It was thus surprising that *Qkb.ksu-4BL.1* was not detected in the cross of HD29 with WH542. However, *Xgwm538* and *Xgwm538snp* were not polymorphic in either cross in this study. The next closest polymorphic marker in both crosses was *Xgwm6*, which is 15–20 cM from *Xgwm538*. This difference in marker coverage compared to the previous study probably decreased our power to detect *Qkb.ksu-4BL.1*. This may explain why *Qkb.ksu-4BL.1* was not detected in WH542/HD29 and explained a relatively small proportion of variation in WH542/W485 in this study. *Qkb.ksu-4BL.1* may be present in several related KB-resistant Indian bread wheat lines; a QTL in the same region was detected in KB-resistant near-isogenic lines (NILs) developed from the cross PBW343/KBRL22 (Seghal 2005). KBRL22, reported to be immune to KB (Sharma et al. 2004), was derived from HD29/W485. The common parentage of HD29 and W485 (both pedigrees contain HD2160; Sharma et al. 2005) supports the speculation that these lines share *Qkb.ksu-4BL.1* by descent.

Although several QTLs were identified in this study, it is likely that additional resistance QTLs remain undetected in these lines. In an earlier report using phenotypic data from F_2, BC_1 and RILs (Sharma et al. 2005), W485 was estimated to carry two resistance genes. The use of an inoculum mixture of 12 diverse isolates, chosen with the intention of identifying broadly effective QTLs, would have reduced the chance of finding isolate-specific QTLs. Uneven chromosome marker coverage, unbalanced marker segregation, and relatively small population sizes further reduced detection power.

Although markers were identified for all three QTLs, these results will require validation for practical application. Studies using NILs and additional mapping populations are in progress.

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