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ORIGINAL PAPER

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Novel quantitative trait loci (QTL) for Fusarium head blight resistance in wheat cultivar Chokwang

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Abstract Fusarium head blight (FHB) is one of the most destructive diseases in wheat. This study was to identify new quantitative trait loci (QTL) for FHB resistance and the molecular markers closely linked to the QTL in wheat cultivar Chokwang. The primers of 612 simple sequence repeats (SSRs) and 12 target-region-amplified polymorphism (TRAP) marker were analyzed between resistant (Chokwang) and susceptible (Clark) parents. One hundred and seventy-two polymorphic markers were used to screen a population of 79 recombinant inbred lines (RILs) derived from the cross of Chokwang and Clark. One major QTL, *Qfhb.ksu-5DL1*, was identified on chromosome 5DL. The SSR marker *Xbarc* 239 was mapped in the QTL region, and also physically located to the bin of 5DL1-0.60-0.74 by using Chinese Spring deletion lines. Another QTL *Qfhb.ksu-4BL1*was linked to SSR *Xbarc* 1096 and tentatively mapped on 4BL. A QTL on 3BS, *Qfhb.ksu-3BS1*, was also detected with marginal significance in this population. Different marker alleles for these QTL were detected between Chokwang and Sumai 3 and its derivatives. These results suggested that Chokwang contains new QTL for FHB resistance that are different from those in Sumai 3. Pyramiding resistance QTL from various sources may enhance FHB resistance in wheat cultivars.

Abbreviations FHB: Fusarium head blight · QTL: Quantitative trait locus · SSR: Simple sequence repeat · TRAP: Target-region-amplified polymorphism · RIL: Recombinant inbred line · IM: Interval mapping · CIM: Composite interval mapping

Introduction

Fusarium head blight (FHB) or head scab of wheat, caused mainly by *Fusarium graminearum*, is one of the economically important diseases of wheat worldwide (Schroeder and Chritensen 1963; Bai and Shaner 2004). FHB causes serious yield loss and deteriorated grain quality when warm and wet weather coincide with anthesis (Bai et al. 1999). Mycotoxin contamination in harvested grain is also a major health concern for human consumption and animal production (Buerstmayr et al. 2002; Bai and Shaner 2004). Fungicide treatments and agriculture management practices may reduce the damage, but yield and quality losses can still be significant in severe epidemic years (Shaner and Buechley 2001). Thus, the use of host genetic resistance is the most efficient approach to diminish the losses caused by FHB.

To date, only a few cultivars have been identified to have a high level of FHB resistance after a large number of germplasm were screened (Snijders 1990; Liu and Wang 1991; Saur 1991; Buerstmayr et al. 2002; Gervais et al. 2003; McCartney et al. 2004). Chinese cultivar Sumai 3 and its derivatives demonstrated the best resistance to FHB and had good combining ability for both FHB resistance and other agronomic traits (Bai and Shaner 2004). Thus, Sumai 3 and its derivatives have been successfully used in breeding programs worldwide (Del Blanco et al. 2003; Somers et al. 2003; Bai and Shaner 2004). However, the extensive use of a single source of resistance may introduce a selection

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pressure on the pathogens to erode the effectiveness of the genes involved (Shaner and Buechley 2001; McCartney et al. 2004). New sources of resistance may broaden the genetic diversity of FHB resistance genes and prevent a potential breakdown of the resistance genes used in current breeding programs.

Wheat resistance to FHB is complex (Bai and Shaner 2004; McCartney et al. 2004). Several types of FHB resistance have been described (Mesterhazy 1995), but mainly type II, resistance to spread of disease symptoms in an infected spike, has been extensively studied (Bai and Shaner 2004). Type II resistance is less prone to environmental effects, which is easier to assess than other types (Buerstmayr et al. 2002), and has been widely used to measure FHB resistance of germplasm and breeding materials (Bai and Shaner 2004).

Coupled with RILs, quantitative trait loci (QTL) mapping is a powerful tool for genetic dissection of a complex trait (Roff 1997). A complex trait can be dissected into several QTL by using molecular markers. Several types of molecular markers have been used to map QTL for FHB resistance (Waldron et al. 1999; Bai et al. 1999; Anderson et al. 2001; Zhou et al. 2003). Among them, simple sequence repeat (SSR) is highly polymorphic, chromosome specific, reproducible, and suitable for automation (Röder et al. 1998); therefore, it is a popular marker system for QTL mapping and gene tagging. A new PCR-based marker system called target region amplified polymorphism (TRAP) has recently been established (Hu and Vick 2003). For TRAP analysis, one primer (fixed primer) is designed based on an expressed sequence tag or other known sequences, and another primer (random primer) is arbitrary with either an AT- or GC-rich core sequence to anneal with an intron or exon, respectively. When used in combination with chromosome-specific markers such as SSRs or RFLPs, TRAP can be assigned to a specific chromosome, and can generate a saturated genetic linkage map (J. D. Faris, personal communication).

Shaner and Buechley (2001) found that Chokwang, a Korean wheat cultivar, demonstrated a high level of type II resistance to FHB, and suggested that QTL conferring resistance to FHB in Chokwang might be different from those in Sumai 3 and its derivatives. The objectives of the present study were to determine chromosomal locations of the QTL conditioning FHB resistance in Chokwang, identify DNA markers closely linked to these QTL, and explore the genetic relationship of the FHB resistance QTL between Chokwang and Sumai 3.

Materials and methods

Plant materials

Chokwang is an FHB-resistant wheat cultivar from Korea (Buechley and Shaner 1999); while Clark is an FHB-susceptible winter wheat cultivar from Purdue University, IN, USA. Two populations of recombinant inbred lines (RILs) were derived from a cross of Chokwang and Clark by single-seed descent at Purdue University. One population with 79 RILs was used for initial QTL identification, and another population with 240 F_6 RILs was used to verify QTLs identified in the first population. Three resistant wheat cultivars from China (Sumai 3, Ning7840, Wangshuibai), one (Ernie) from the USA, and two susceptible wheat cultivars (Petterson and Wheaton) from the USA were selected for haplotype analysis with selected SSR markers linked to putative QTLs for FHB resistance identified in this study.

FHB evaluation

F_7 and F_8 populations of 79 RILs were evaluated for spread of FHB symptoms within a spike in the greenhouse at Purdue, in 1999 and 2000, respectively. Generations F_{10} and F_{11} were evaluated in the greenhouse of Kansas State University, Manhattan, KS, USA, in 2003 and 2004, respectively. Plant growth and FHB evaluation followed the procedure of Bai et al. (1999). For each experiment, three replications (pots) were evaluated for type II resistance with three to six plants in each replication. In 1999 and 2000, scabbed spikelets were counted 3, 9, 15, and 21 days after inoculation. These data were used to calculate the area under the disease progress curves (AUDPC) for each inoculated spike, according to Shaner and Finney (1977). Because a good correlation was observed between AUDPC and percentage of scabbed spikelets (PSS) on the 21st day after inoculation ($R = 0.95$, $P < 0.01$, 1999; and $R = 0.96$, $P < 0.01$, 2000), PSS on the 21st day after inoculation was evaluated in 2003 and 2004 experiments.

To verify identified QTL in a larger population, a population with 240 F_6 RILs derived from the cross between Chokwang and Clark was evaluated for type II resistance in the greenhouses of Kansas State University in the fall of 2004. PSS on the 21st day after inoculation was calculated for the population.

SSR analysis

Wheat leaves were harvested from 15 plants of each F_{10} line the day before the plants were transplanted into a greenhouse. The tissues were dried in a freeze dryer (ThermoSavant, Holbrook, NY, USA) for 48 h, and ground by a Mixer Mill (MM 300, Rotsch Inc, Germany). A CTAB protocol was used for isolation of genomic DNA (Saghai-Maroof et al. 1984).

A collection of 612 microsatellite primers was screened for polymorphism between Chokwang and Clark. These primers included 460 BARC primers (Song et al. 2005), 30 GWM primers (Röder et al. 1998), 70 CFA primers, and 58 CFD primers (Guyomarc'h et al. 2002; Sourdille et al. 2003). Primers that amplified at least one polymorphic band between parents were used

to screen the RILs. For SSR marker analysis, an M13 tail sequence (5′-ACGACGTTGTAAAACGAC) was attached to the 5′-end of each forward primer. The PCR reaction was performed in 10-μl volumes including 50 ng of template DNA, 0.6 U of *Taq* DNA polymerase (Promega, WI, USA), 1× PCR buffer, 2.5 mM $MgCl_2$, 0.25 μM of each dNTP, 0.2 μM fluorescence-labeled M13 primer, and 0.1 μM of each of the regular primers. The reaction mixture was denatured at 94°C for 5 min, followed by 5 cycles at 94°C for 45 s, 68°C for 5 min with a decrease of 2°C in each subsequent cycle, and 72°C for 1 min; followed by 5 cycles at 95°C for 45 s, and 58°C for 2 min with a decrease of 2°C in each subsequent cycle, and 72°C for 1 min; 25 cycles at 94°C for 45 s, 50°C for 2 min, and 72°C for 1 min; and a final extension step of 72°C for 4 min. PCR products were resolved in an ABI Prism 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Data were analyzed using GeneMapper software (Version 3.5, Applied Biosystem, Foster City, CA, USA).

TRAP analysis

Sequences of random primers from three sources (Table 1) were labeled with IR-dye 700 or IR-dye 800 at the 5′-end for PCR detection. For fixed primers, HF2 and BF1 were adopted from Hu and Vick (2003) and Li and Quiros (2001), respectively, and the other two were designed based on sequences of two wheat ESTs (http://wheat.pw.usda.gov/cgi-bin/westsql/map-locus.cgi by using the Primer 3 software (Rozen and Skaletsky 2000). Amplification and detection of PCR followed the procedure of Hu and Vick (2003). The names of TRAP markers were designated as: random primer-fixed primer-DNA fragment size.

Physical mapping of *Xbarc* 239 marker

SSR marker *Xbarc* 239 was the closest marker to a major QTL for FHB resistance found in this study, but its physical location was not determined previously. To physically map this marker, a ditelosomic line without the 5D long arm (ditelosomics of the 5D short arm are not available. Sears and Sears 1978) was analyzed with the SSR marker. A set of seven deletion lines of chromosome 5D was used to locate the sub-arm location of the SSR marker (Endo and Gill 1996).

Data analysis

RILs and their parents were arranged in a completely randomized design for FHB evaluation in the greenhouse. SAS program version 8.1 (SAS Institute Inc. Cary, NC, USA) was used for analyses of phenotypic data, including analysis of variance and correlation. The SAS program "PROC GLM" was used to estimate the joint R^2 value of markers closely linked to the QTLs. The heritability across the four years' experiments was estimated with the formula $h^2 = \sigma^2_G/[\sigma^2_G + (\sigma^2_{GE}/E) + (\sigma^2_e/rE)]$, where σ^2_G is the genetic variance; σ^2_{GE} is the variance for genotype by environment interaction; σ^2_e is the residual variance; E is the number of environments and r is the average number of plants per line across the four experiments.

JoinMap version 3.0 (Van Ooijen and Voorrips 2001) was used to construct a genetic linkage map. The threshold value of LOD (logarithm of odd) score was set at 3.0 to claim linkage between markers with a maximum fraction of recombination at 0.4. Recombination fractions were converted into centiMorgans (cM) by using the Kosambi function (Kosambi 1944). The goodness-of-fit between observed and expected segregation ratios at both loci were analyzed by using a Chi-square test. For QTL analysis, interval mapping (IM) and composite interval mapping (CIM) were performed with QTL Cartographer Version 2 (Basten et al. 2003). Both separated and combined analyses of phenotypic data from 4 years were presented. The threshold for significant QTL was determined by a 1,000-permutation test (Basten et al. 2003).

Results

FHB phenotype

The two parents, Chokwang and Clark, showed a significant difference in FHB spread within a spike (type

Table 1 Fixed and random primer designations, primer sequences, source of EST accessions and the species that the ESTs derived from, and labeled IR dyes used in this study

	Sequence	Source		IR dye
		EST	Species	
Fixed primer				
HF2[a]	CGTTTATTTCCTCGCCTC	B18I19b	*H. annuus* L.	N/A
BF1[b]	TGAGTCCAAACCGGATA	Unknown	*B. rapa* L.	N/A
5WF3	AAATCGGCGAACGACACA	BE499071	*T. aestivum* L.	N/A
4WF1	GCCTTCGTCTACGTGAGTCC	BG274947	*T. aestivum* L.	
Random primer				
HR1[a]	GGAACCAAACACATGAAGA	N/A	*H. annuus* L.	700
BR3[b]	GACTGCGTACGAATTGAC	N/A	*B. rapa* L.	700
WR6[c]	ACGTCTGATCAGGCCGTA	N/A	*T. aestivum* L.	800

[a]Adopted from Hu and Vick (2003)
[b]Adopted from Li and Quiros (2001)
[c]Adopted from J. D. Faris, personal communication

II resistance) in the greenhouse conditions. Chokwang had a low PSS of 10.7% in 2003 and 16.7% in 2004, whereas Clark had PSS as high as 88.5% in 2003 and 96.5% in 2004. Mean PSSs for RILs were 54.3% in 2003 and 65.2% in 2004, with a wide range of segregation for PSS among RILs (Fig. 1). Lines with significantly greater resistance than that of Chokwang were not found. The frequency distribution of PSSs averaged over the four experiments was bimodal (Fig. 1), similar to those of AUDPCs observed previously (Shaner and Buechley 2001). The correlations of disease severities of RILs were highly significant among the four experiments ($P < 0.0001$), with R-values ranging from 0.36 (between 2003 and 2004) to 0.57 (between 1999 and 2000). Significant variations were observed for genotypes, years and interaction between genotypes and years (Table 2). Broad-sense heritability was about 0.69.

QTL analysis

Initial screening of putative QTL using IM detected four QTLs (Table 3). Among them, *Qfhs.ksu-5DL1* on chromosome 5D demonstrated the lowest FHB severity with an average PSS of 24.3%. This QTL was significant ($P < 0.05$) for the data from 2004 and was highly significant ($P < 0.01$) for data from 1999, 2000, 2003 and for the data averaged over 4 years (Table 3, Fig. 2a). Another putative QTL was detected about 23.7 cM away from *Qfhs.ksu-5DL1*, and accounted for 10.5% (2004) to 21.5% (2000) of phenotypic variations. The *Qfhs.ksu-4BL1* on chromosome 4BL explained 10.6% (2003) to 15.9% (2000) of phenotypic variation and was highly significant in 2000 and significant for data from 1999, 2003, 2004 and for the data averaged over 4 years (Fig. 3). One QTL on 3B, *Qfhs.ksu-3BS1*, was only highly significant in 2004 and significant in 1999. On the basis of the estimates of the four closest SSR markers by multiple regressions, joint contribution of these QTLs was from 32 to 36% of the total phenotypical variation for FHB resistance.

To further verify these putative QTL, CIM was also conducted for the data by selecting markers closely linked to QTLs detected by IM as cofactors. Results from CIM gave slightly lower LOD values for the QTLs than those from IM (Table 3). In CIM, QTL *Qfhs.ksu-5DL1* was still significant in all four experiments, but the second putative QTL on 5DL was not significant. The QTL *Qfhs.ksu-4BL1* was significant only for data from 2000. The *Qfhs.ksu-3BS1* QTL was only marginally significant in 1999.

To validate the marker association with the QTL in a larger population, a F_6 population of 240 RILs derived from the same cross was analyzed for FHB resistance and genotyped with the markers closely linked to the target QTL. A total of nine markers from three QTL regions on 5DL (Barc239, Cfd57 Cfd3), 4BL (Barc1096, Barc185, Barc20) and 3BS (Gwm533, Gwm389, Gwm493) were selected to genotype the population, and five markers were significantly associated with the three QTL for FHB resistance. Single marker regression analysis indicated that markers *Xbarc* 239, *Xcfd* 57, *Xbarc* 1096, *Xbarc* 185, and *Xgwm* 533 explained 19.4, 7.3, 8.4, 7.2 and 4.9% of phenotypic variations, respectively. This result suggests that QTL on chromosomes 5DL, 4BL and 3BS are true QTL for FHB resistance in Chokwang.

Confirmation of physical location of *Qfhs.ksu-5DL1*

Qfhs.ksu-5DL1 is a new QTL reported in this study, and it demonstrated a major effect on FHB resistance in the mapping population (Table 3). SSR markers *Xcfd* 3, *Xcfd* 57 and *Xbarc* 361, which had been previously located on 5DL (Shi et al. 2003; Somers et al. 2004), were mapped in the QTL region in this study (Fig. 2). But the chromosome location of *Xbarc* 239, the closest marker to the QTL, has not been mapped before. *Xbarc* 239 amplified a co-dominant marker with a 313-bp fragment in Chokwang and a 301-bp fragment in Clark. Chinese Spring amplified the same band as Chokwang. All nullitetrasomic lines showed the target band except N5D, confirming that the location of *Xbarc* 239 is on chromosome 5D. When a 5DL ditelosomic line and eight deletion lines of chromosome 5D were screened with *Xbarc* 239, the 313-bp band was absent in the 5DL ditelosomic line and 5DL deletion lines of 5DL1 and

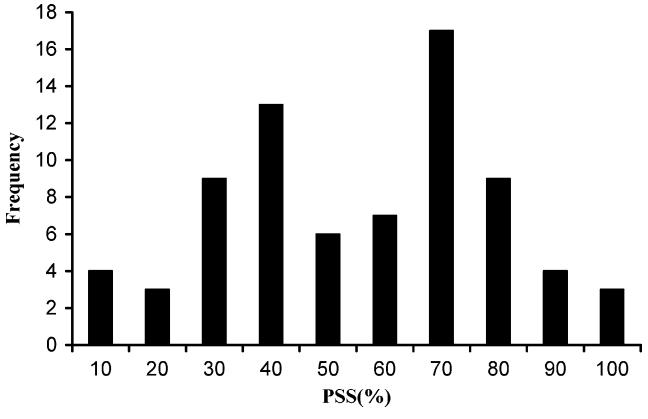

Fig. 1 Frequency distribution of FHB severity (PSS) for wheat RILs derived from a cross of Chokwang and Clark. Data are averaged over four experiments (1999, 2000, 2003 and 2004)

Table 2 Analysis of variance for percentage of scabbed spikelets (PSS) evaluated in the RILs from the cross of Chokwang and Clark based on the data from four experiments

Variables	*df*	MS	*F*-value	*P*-value
RIL	78	5318.1	6.25	< 0.01
Year	3	34797.3	40.88	< 0.01
RIL × Year	234	3127.3	3.67	< 0.01
Error	1,423	851.1		

Table 3 Name of QTLs, linked markers, LOD values and coefficients of determination (R^2) estimated by interval mapping (IM) and composite interval mapping (CIM) in the RILs derived from a cross of Chokwang and Clark for four experiments and their averages

Locus	Close marker	1999		2000		2003		2004		Average	
		LOD	R^2	LOD	R^2	LOD	R^2	LOD	R^2	LOD	R^2
IM											
Qfhs.ksu-5DL1	*Xbarc* 239	3.58	29.60	5.15	31.84	2.72	25.26	2.28	18.88	3.32	24.30
Qfhs.ksu-5DL2	*Xcfd* 3	2.26	18.8	3.53	21.5	1.85	15.7	1.75	10.5	2.27	16.63
Qfhs.ksu-4BL1	*Xbarc* 1096	2.23	15.56	2.84	15.89	1.84	10.57	1.81	12.00	2.08	13.21
Qfhs.ksu-3BS1	*Xgwm* 533	2.15	11.99	0.74	6.82	0.86	6.98	2.18	13.39	1.57	9.80
CIM											
Qfhs.ksu-5DL1	*Xbarc* 239	2.42	11.26	2.93	13.26	1.84	9.04	1.90	8.98	2.16	10.54
Qfhs.ksu-4BL1	*Xbarc* 1096	0.70	3.08	2.26	10.37	0.73	3.52	0.54	2.46	1.11	4.67
Qfhs.ksu-3BS1	*Xgwm* 533	1.78	6.82	n	n	0.69	3.50	1.10	8.60	1.09	6.02

Threshold value of LOD was 1.8 at a significance level $P < 0.05$ and 2.31 at significance level $P < 0.01$, determined by a 1,000-permutation test

n none allele was detected

5DL7, but was present in all other deletion lines (Fig. 4). Therefore, *Xbarc* 239 was physically located in the bin of 5DL1-0.60-0.74 (Qi et al. 2003; Sourdille et al. 2004), which agrees with the result from the genetic mapping.

On the basis of the available SSR mapping information, other QTLs were also tentatively assigned to different chromosome arms. The *Qfhs. ksu-4BL1* QTL was assigned to chromosome 4BL because its linked markers *Xbarc* 1096 and *Xbarc* 20 were assigned to that linkage group (Shi et al. 2003; Somers et al. 2004; Sourdille et al. 2004), and QTL *Qfhs.ksu-3BS1* was assigned to the short arm of chromosome 3B due to its linkage to markers *Xgwm* 389, *Xgwm* 493 and *Xgwm* 533 (Röder et al. 1998).

Haplotype analysis of SSR markers linked to QTLs for FHB resistance

Allele sizes of PCR products that were amplified from three SSR primers on 3BS (GWM389, GWM493, and GWM533), one primer on 4BL (BARC1096), and two primers on 5DL (BARC239, CFD3) were compared among eight cultivars of various origins or with different levels of FHB resistance (Table 4). The PCR products

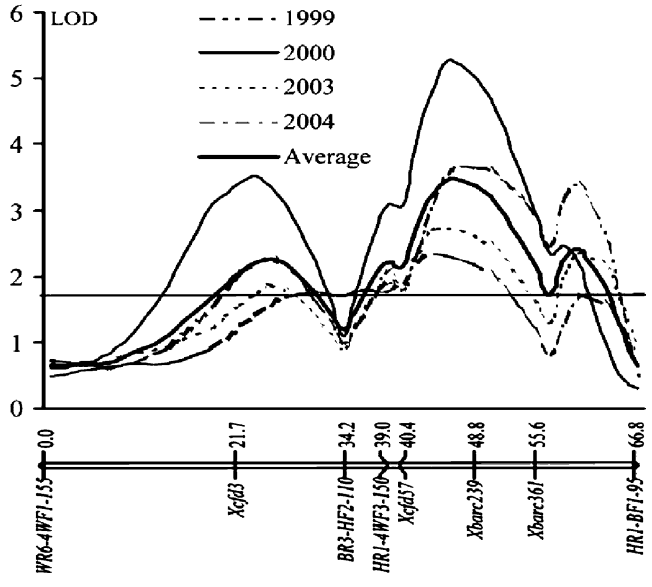

Fig. 2 Linkage and QTL maps of chromosome 5DL constructed with SSR and TRAP markers from the RIL population derived from cross of Chokwang and Clark to show the result from IM for four experiments (1999, 2000, 2003 and 2004) and their mean. In the graph, the QTL map is on the *top* and the linkage group is on the *bottom*. The *solid line* parallel to the *X*-axis is the threshold line for LOD 1.8 ($P < 0.05$). Genetic distances are shown in centiMorgans (cM) above the linkage group, and markers are below the linkage group

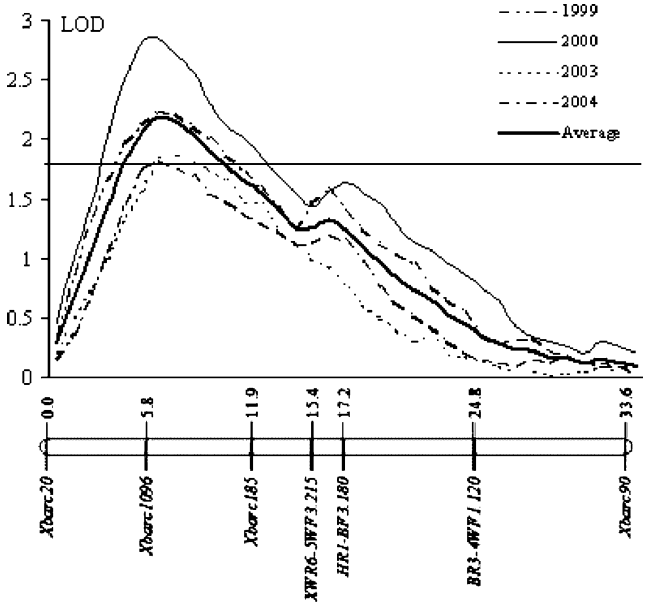

Fig. 3 Interval mapping (*IM*) of QTL on chromosome 4BL with SSR and TRAP markers using phenotypical data from four experiments (1999, 2000, 2003 and 2004) and their grand mean. QTL map is on the *top* and linkage group is on the *bottom*. The *solid line* parallel to *X*-axis is the threshold line for LOD 1.8 ($P < 0.05$). Genetic distances are shown in centiMorgans (cM) above the linkage group, and markers are below the linkage group

that were amplified from Chokwang by the three 3BS primers showed different sizes from those in Sumai 3, Wangshuibai, and four US cultivars except that primer GWM493 amplified the same allele as in Ning 7840 and Sumai 3. In addition, PCR products amplified from Chokwang by the other three primers differed from those in Sumai 3 and the other cultivars except Peterson and Ernie.

Discussion

To date, three sources of FHB resistance have been identified: spring wheat from Asia, spring wheat from Brazil, and winter wheat from Europe and North America (McCartney et al. 2004). The Asian source includes a large collection of FHB-resistant materials mainly from China and Japan. Most of them are landraces; some are improved cultivars. Sumai 3 and its derivatives from China have been recognized as the most resistant resource for FHB (Bai and Shaner 2004). A major QTL on 3BS from Sumai 3 has been used extensively in breeding programs worldwide, and is common in many Japanese resistant materials. Therefore, the identification of the resistance genes/QTL from sources other than Sumai 3 may broaden the genetic diversity of FHB resistance and prevent breakdown of FHB resistance in the future. Chokwang, a Korean winter wheat, displayed a low AUDPC, and was considered a new source of FHB resistance (Shaner and Buechley 2001). The result from the present study confirmed this conclusion. The bimodal frequency distributions of PSSs and AUDPCs observed in the RIL population suggested a major genetic effect of genes/QTL on FHB severity in Chokwang. A similar distribution pattern has also been reported in several other wheat populations (Bai et al. 1999; Waldron et al. 1999; Buerstmayr et al. 2002), in which one QTL with a major effect on FHB resistance was identified on chromosome 3BS.

QTL mapping indicated that Chokwang had one QTL (*Qfhs.ksu-5DL1*) exerting a major effect on FHB resistance (Table 3). This QTL was consistently detected among all experiments (Fig. 2), and explained 19–32% of phenotypic variations for FHB severity in the four experiments. To verify the QTL, another population of 240 RILs derived from the cross between Chokwang and Clark was tested in the greenhouse of 2004. The result indicates that marker *Xbarc* 239 can explain 19% of phenotypic variation ($P < 0.01$). This QTL may have a similar effect on FHB resistance as that of the 3BS QTL

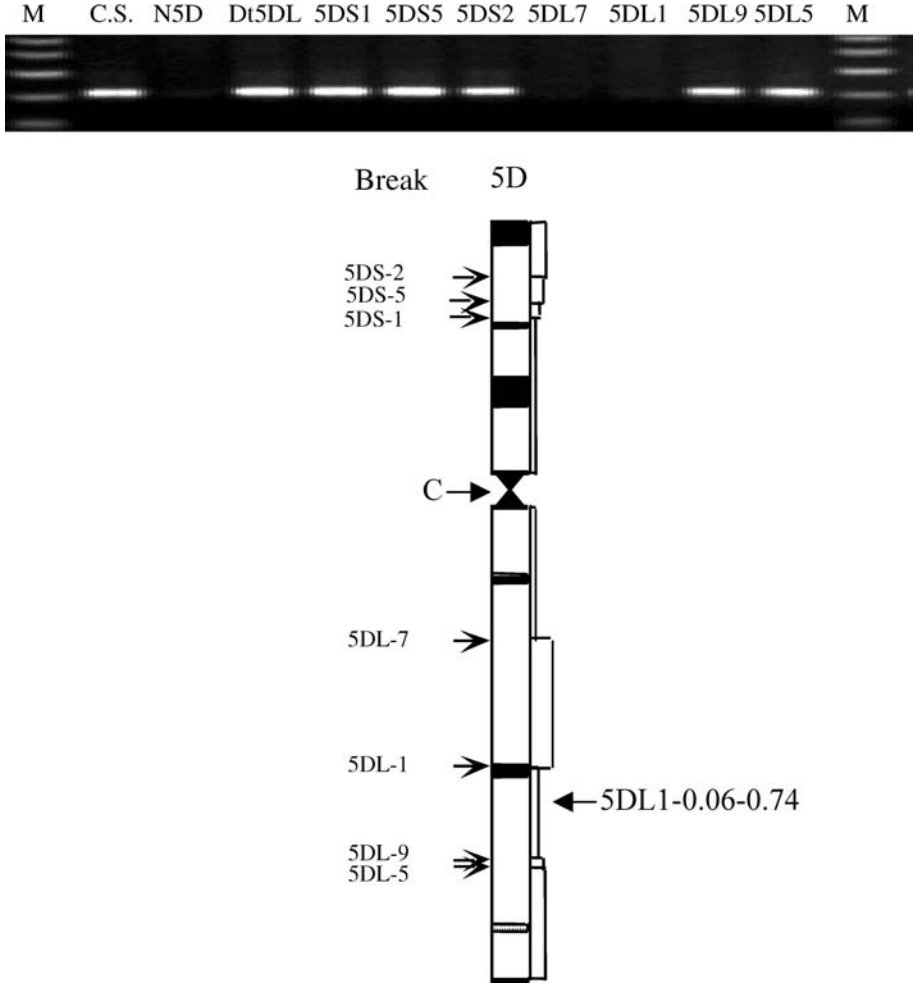

Fig. 4 *Top* Electropherogram of PCR products amplified by SSR *Xbarc 239* on an agarose gel (2% agarose, 80 V, 1 h), where *M* is 100-bp PCR marker, C.S. is wheat var. Chinese Spring, and the rest are Chinese spring aneuploids for chromosome 5D. *Bottom* is an ideogram of wheat Chromosome 5D where the *arrows at left* indicate break points of deletion lines, and *C* indicates the centromere

Table 4 Allele sizes (bp) for six SSR markers in eight wheat cultivars

	Xgwm 389	*Xgwm* 493	*Xgwm* 533	*Xbarc* 1096	*Xbarc* 239	*Xcfd* 3
Chokwang	136	192	134	172	313	172
Sumai 3	140	192	140	162	301	180
Wangshuibai	138	194	136	172	316	180
Ning7840	139	192	138	172	316	182
Erine	114	137	137	172	313	n
Wheaton	113	137	112	172	n	180
Clark	113	156	112	162	301	180
Peterson	113	190	111	131	313	172

The six SSR markers were linked to four putative QTLs (Table 2) detected in the genome of wheat cultivar Chokwang
n no allele was detected

from Sumai 3, which reportedly contributed 15.4–60% of phenotypic variation for FHB resistance (Waldron et al. 1999; Bai et al. 1999; Anderson et al. 2001; Buerstmayr et al. 2002; Del Blanco et al. 2003). A minor QTL ($R^2 = 4\%$) was reported on 5DL in a RIL population derived from the cross between two European winter wheat cultivars Renan and Recital (Gervais et al. 2003), but the QTL was located in a different region of the chromosome from the one identified in this study. SSR marker *Xbarc* 29 was closely linked to the minor QTL in their study and was located in the bin of 5DL5-0.76-100 (Shi et al. 2003; Qi et al. 2003; Sourdille et al. 2004), while *Xbarc* 239 was linked to *Qfhs. ksu-5DL1* in this study, but was assigned in the bin of 5DL1-0.60-0.74 (Fig. 4). Therefore, *Qfhs.ksu-5DL1* is a novel QTL. It is noticed that a lower determination coefficient value was observed from QTL validation population than that from original small mapping population. This may be due to the small population size of the original mapping population that exaggerates the contribution of the QTL. However, it showed major effect on both populations in repeated tests; it is a stable QTL for type II resistance.

The genetic distance between *Qfhs.ksu-5DL1* and its closest SSR marker, *Xbarc* 239, was about 2.5–5.6 cM (Fig. 2). *Xbarc* 239 should be a good marker for marker-assisted selection and for pyramiding FHB resistance with the QTLs from other sources. For molecular cloning of the QTL, however, fine-mapping this QTL is necessary. A recent study showed that 5DL1-0.60-0.74 is an EST-rich bin with 83 ESTs mapped in it (Qi et al. 2004). Given that *Qfhs. ksu-5DL1* is in the same bin as *Xbarc* 239, two strategies can be taken to implement fine mapping of the QTL. The first is to use the deletion lines 5DL-1 and 5DL-9 to screen potential DNA markers, both random- and non-random-based. The second is to develop new STS markers, such as EST-STS, on the basis of the information generated from the chromosome bin map.

Besides the major QTL on 5DL, three other QTL were also detected in this study. A second QTL on 5DL was detected in three out of four experiments by IM, but not detected by CIM. Because CIM adds background loci to IM to check the effect of these loci on the target QTL, CIM could remove the bias that would normally be due to QTL linked to the position being tested (Basten et al. 2003). In addition, the QTL for FHB resistance was not significant in single marker analysis of the larger population when *Xcdf*3 was used as the linked marker. Therefore, the second QTL on 5DL is most likely a false positive caused by the linked major QTL *Qfhs.ksu-5DL1*. The QTL *Qfhs.ksu.3BS1* on 3BS was also identified by both IM and CIM in most of the experiments, and it mapped to the same location as the 3BS major QTL in Sumai 3. Unlike the 3BS QTL in Sumai 3, however, the QTL in Chokwang showed much smaller effects on FHB resistance, and linked SSR markers exhibited different banding patterns from those in Sumai 3. It is possible that the 3BS QTL in Chokwang is at the same locus as in Sumai 3, but is a different allele.

Somers et al. (2003) reported a QTL on 4B that explained 12% of phenotypic variation in field conditions and that it was linked with SSR marker *Xgwm* 107 and derived from a Chinese resistant wheat accession, Wuhan-1. In this study, QTL *Qfhs.ksu-4BL1* was also found on 4BL and explained 8.4–15.9% of phenotypic variation (Table 3). The closest marker to the QTL of our study was *Xbarc* 1096, which mapped in the chromosome region proximal to the centromere of 4BL. The marker *Xgwm* 107 mapped in the same region (Shi et al. 2003), suggesting that *Qfhs.ksu-4BL1* in Chokwang may be the same QTL found in Wuhan-1. They could be either the same or different alleles of the same locus.

Chokwang and Sumai 3 may have the same QTL on 3BS for type II resistance, but the QTL on 5DL and 4BL in Chokwang have not been reported in Sumai 3. On the contrary, additional resistant QTL reported in Sumai 3 on chromosome 5A (Buerstmayr et al. 2002), and 6B and 6A (Anderson et al. 2001) have not been found in Chokwang. This indicates that the QTL for FHB resistance in Chokwang is different from that in Sumai 3. Moreover, haplotype analysis (Table 4) revealed that the resistant QTLs in Chokwang were mostly different from those found in the North American cultivars. Thus, genes in Chokwang could be useful for FHB-resistance QTL stacking in wheat-breeding programs and for further genetic analysis. Increased effectiveness of FHB resistance by pyramiding more than one FHB-resistance QTL has already been demonstrated in wheat cultivar Sumai 3.

In this study, TRAP marker was used in attempt to saturate QTL regions. TRAP markers were expected to detect more polymorphism than EST-STS and to be more chromosome specific than AFLP (Hu and Vick 2003). It combines one random primer with a chromosome-specific primer designed based on a specific EST sequence. Although TRAP markers significantly increased map density in this study, it failed to add any new markers to the QTL peak region as expected (Fig. 2). This could be due to the fact that the selected wheat ESTs for fixed primer was still far from the target QTL. It is also possible that TRAP primers amplified polymorphic PCR products from a genomic region different from the expected region where fixed primer was from. This may not be surprising considering the complex genome structure of wheat. Therefore, use of TRAP as target markers for fine mapping of a specific chromosome region may need to be cautious.

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