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Saturation and comparative mapping of a major Fusarium head blight resistance QTL in tetraploid wheat

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Abstract Fusarium head blight (FHB) is a devastating disease of cultivated wheat worldwide. Partial resistance to FHB has been identified in common wheat (*Triticum aestivum* L.). However, sources of effective FHB resistance have not been found in durum wheat (*T. turgidum* L. var. *durum*). A major FHB resistance quantitative trait loci (QTL), *Qfhs.ndsu-3AS*, was identified on chromosome 3A of *T. dicoccoides*, a wild relative of durum wheat. Here, we saturated the genomic region containing the QTL using EST-derived target region amplified polymorphism (TRAP), sequence tagged site (STS), and simple sequence repeat (SSR) markers. A total of 45 new molecular marker loci were detected on chromosome 3A and the resulting linkage map consisted of 55 markers spanning a genetic distance of 277.2 cM. *Qfhs.ndsu-3AS* was positioned within a chromosomal interval of 11.5 cM and is flanked by the TRAP marker loci, *Xfcp401* and *Xfcp397.2*. The average map distance between the marker loci within this QTL region was reduced from 4.9 cM in the previous study to 3.5 cM in the present study. Comparative mapping indicated that *Qfhs.ndsu-3AS* is not homoeologous to *Qfhs.ndsu-3BS*, a major FHB QTL derived from the common wheat cultivar Sumai 3. These results facilitate our efforts toward map-based cloning of *Qfhs.ndsu-3AS* and utilization of this QTL in durum wheat breeding via marker-assisted selection.

Keywords Comparative mapping · Fusarium head blight · QTL · Saturation mapping · *Triticum*

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Introduction

Fusarium head blight (FHB), caused primarily by the fungus *Fusarium graminearum* Schwabe [teleomorph *Gibberella zeae* (Schw.) Petch], is a devastating disease of both common wheat (*T. aestivum* L., $2n = 6x = 42$, genomes AABBDD) and durum wheat (*T. turgidum* L. var. *durum*, $2n = 4x = 28$, genomes AABB) worldwide. Epidemics of this disease can cause significant economic losses in terms of yield and quality in wheat. Host resistance has been considered an economically efficient and environmentally sounds measure to manage this destructive disease. Sources of

FHB resistance currently identified in wheat exhibit complex quantitative inheritance patterns. FHB resistance quantitative trait loci (QTL) have been detected in common wheat (Waldron et al. 1999; Kolb et al. 2001; Bai and Shaner 2004; Mardi et al. 2005; Yang et al. 2005). A major FHB resistance QTL *Qfhs.ndsu-3BS* derived from the Chinese common wheat cultivar Sumai 3, a widely used source of resistance in wheat, has been extensively characterized, including physical mapping, fine mapping and comparative mapping (Waldron et al. 1999; Buerstmayr et al. 2002; Liu and Anderson 2003a, b; Liu et al. 2006).

Sources of effective FHB resistance have not been found in durum wheat. Screening of a set of disomic substitution lines (LDN-DIC), where one pair of homologous chromosomes from the wild emmer wheat [*T. dicoccoides* (Körn. ex Asch. & Graebner) Thell., $2n = 4x = 28$, genomes AABB] accession Israel-A substituted for the corresponding homologous chromosome pair of the durum wheat cultivar Langdon, indicated that chromosome 3A of Israel-A carried a gene conditioning Type II resistance to FHB (resistance to the spread of FHB infection) (Stack et al. 2002). Otto et al. (2002) identified a major FHB resistance QTL on chromosome 3A designated as *Qfhs.ndsu-3AS*, which explained 37% of the phenotypic variation. A linkage map of the QTL was constructed using a population of 83 recombinant inbred chromosome lines (RICLs) derived from the cross between LDN and LDN-DIC 3A. *Qfhs.ndsu-3AS* was positioned within a 29.3 cM chromosomal interval (Otto et al. 2002).

Various molecular marker techniques such as restriction fragment length polymorphism (RFLP), simple sequence repeat (SSR), sequence tagged site (STS), and amplified fragment length polymorphism (AFLP) have been used for saturation mapping in wheat and other plants (Faris et al. 2000; Liu and Anderson 2003b; Nguyen et al. 2004). A recently developed PCR-based marker technique by Hu and Vick (2003) known as target region amplified polymorphism (TRAP) was shown to be a reliable and robust marker platform for genetic mapping in wheat (Liu et al. 2005; Yang et al. 2005). The PCR-based technique relies on the use of an arbitrary primer in combination with 'fixed' primers designed from expressed sequence tags (ESTs). Because more than 16,000 EST loci have been physically mapped to chromosomal deletion bins in wheat (Qi et al. 2004), the number of potential primer combinations for generating TRAP markers is virtually unlimited.

The objectives of this study were to saturate the chromosomal region harboring *Qfhs.ndsu-3AS* with molecular markers, to refine the map position of the QTL, and to determine if *Qfhs.ndsu-3AS* and *Qfhs.ndsu-3BS* resided at homoeoloci. This work led to the development of effective markers to assist selection of the QTL in breeding programs. The markers will also facilitate cloning of the QTL, which is necessary to gain better understanding of the molecular mechanism underlying FHB resistance.

Materials and methods

Plant materials

The population of 83 RICLs used for saturation mapping was developed from the cross between LDN and LDN-DIC 3A by Joppa (1993). Otto et al. (2002) used this population in the identification and initial mapping of the FHB resistance QTL *Qfhs.ndsu-3AS*. We used this population for saturation and comparative mapping of the QTL in this study. The LDN-DIC 2A and 3A involved in this study were developed by Williams and Joppa (1988). All the materials were grown in a greenhouse to collect leaf tissues for DNA extraction.

FHB resistance evaluation and statistical analysis

Reactions of the 83 RICLs to FHB infection were evaluated over two seasons in greenhouses with controlled environments. During both seasons, artificial lighting was employed to provide a 16 h photoperiod. Temperature was maintained at approximately 27°C. LDN and LDN-DIC 2A were used as susceptible controls and LDN-DIC 3A and the common wheat cultivar Sumai 3 as resistant controls in the evaluation experiments. Plants were grown in pots (6.5 inch in diameter

and 7.0 inch in height) filled with Sunshine SB100 Mix (Sun Gro Horticulture Distribution Inc., Bellevue, WA), and fertilized with Osmocote Plus 15-19-12 (Scotts Sierra Horticultural Product Company, Marysville, OH) in a greenhouse and arranged in a randomized complete block design with three replicates in both seasons. Each replicate consisted of two plants per pot. Artificial lighting was employed to provide a 16 h photoperiod. Temperature was maintained at approximately 27°C. An average of 10 spikes per replicate was inoculated. Inoculation was performed following the methods described by Stack et al. (2002). Briefly, *Fusarium graminearum* cultures were grown on half strength potato dextrose agar in the laboratory. To account for variations in resistance to different isolates, three strains of pathogenic *F. graminearum* were used. Inoculum was prepared by flooding the cultures with sterile distilled water and straining the resulting suspension through sterile cheesecloth. The final conidiospore suspension was adjusted to a concentration of 50,000 spores ml^{-1}. Ten microliters of the suspension was injected into a single central spikelet per spike at anthesis. To facilitate disease development and increase the stringency of the evaluation, humidity was maintained for 72 h post-inoculation by covering each spike with a plastic bag and misting at least once daily. Disease severity (Type II resistance) was visually scored as the percentage of diseased spikelets in a spike at 21 days post inoculation.

Analysis of variance (ANOVA) and regression and correlation analysis of phenotypic data were performed using the Statistical Analysis System version 8.2 (SAS Institute 1999).

SSR and STS analysis

Total genomic DNA was isolated from the RICLs, LDN, and LDN-DIC 3A using the method described by Faris et al. (2000). SSR PCR was performed as described by Röder et al. (1998). STS PCR followed the procedures described by Liu and Anderson (2003b). PCR products amplified from both STS and SSR marker loci were differentiated on 6% non-denaturing polyacrylamide gels. The gels were stained by ethidium bromide and visualized under UV light.

The STS and SSR primers were designed based on ESTs assigned to the chromosomal deletion bin 3AS 0.45–1.00, which contains the QTL *Qfhs.ndsu-3AS* (http://wheat.pw.usda.gov/cgi-bin/westsql/map_locus.cgi). Nucleotide-binding site (NBS) and/or leucine-rich repeat (LRR) sequences have been found in many disease resistance genes and some kinase-like genes confer disease resistance in plants (Ayliffe and Lagudah 2004). One hundred and sixty-five ESTs residing in this chromosome bin were subjected to BLASTx (Altschul et al. 1997) alignments to sequences in the NCBI nonredundant (nr) public database (http://www.ncbi.nlm.nih.gov/) to identify NBS and/or LRR domains. ESTs with these domains were used to design STS primers. The 5′-sequences of all the ESTs mapped in this chromosome bin were searched for SSRs using simple sequence repeat identification tool (SSRIT) (Temnykh et al. 2001; http://www.gramene.org/db/searches/ssrtool). For the ESTs from which no STS or SSR markers were developed through the above approaches, we used the consensus contig sequences of those ESTs, if available, and the sequences of the individual ESTs to design 100 pairs of STS primers. All the SSR and STS primers were designed using the web-based primer designing program Primer 3 (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi; Rozen and Skaletsky 2000). Primer sequences of the new STS markers mapped on chromosome 3A in this study are listed in Table 1.

TRAP analysis

TRAP marker analysis was conducted as described by Hu and Vick (2003). A single fixed primer in combination with two random primers each labeled by a different dye (IR-700 or IR-800, Li-Cor Biosciences, Lincoln, NE) were used in the PCR. Fixed primers of the TRAP markers included those designed from the ESTs containing NBS-LRR domains and the EST-derived SSR and STS primers used in this study. Both fixed and random primers of the TRAP markers assigned to chromosome 3A are listed in Table 2. The PCR products were visualized on a Li-Cor Global DNA Sequencer 4300. Electrophoresis was conducted at 2,000 V, 40 mA, 50 W for 2.5 h.

Table 1. Primer sequences of the wheat EST-derived STS markers mapped on chromosome 3A

Marker	Primers (5′ → 3′)	Annealing Temperature (°C)	Source*
Xfcp408	F: AAGACAGCCCTCAAGGATGA R: CAAGCGCATATGGATGACAG	58	BE637850
Xfcp396	F: TACCATCCTGCTGATGCTTG R: CACTCGAAGACCTGGTCCAT	58	BE425222
Xfcp402	F: GGTCCAAGCTTTCAGTCGAG R: TGGTTAGGGAACCAACTCCA	58	BE517736
Xfcp399	F: GGCGCTGATCTATCATCCAT R: CACATTGGTCCTCCACACAG	58	BF484475
Xfcp420	F: CTCAAGATGGGTTGGTGGTT R: CGAGGCCCCTTCTACTCTCT	58	BE498100

* The GenBank wheat EST accession number

Table 2. Primer sequences of the TRAP markers mapped on chromosome 3A

Marker	Random primer (5′ → 3′)	Fixed primer (5′ → 3′)	Source of the fixed primer*
Xfcp417	CGTAGTGATCGAATTCTG	ATCTGCTTGAAGGTGTGC	BE489782
Xfcp395	TTCTAGGTAATCCAACAACA	CAGGTATGAAATCGCCCCTA	BJ305082
Xfcp409	TTCTAGGTAATCCAACAACA	TTGCAGCTGGAATCACTACC	BE405200
Xfcp398	CGTAGTGATCGAATTCTG	GACGACATGTTCTCGGAGGT	BF483989
Xfcp413	GCGCGATGATAAATTATC	TGATTTTCTTCGCCTCTTGG	BE404719
Xfcp405	GCGCGATGATAAATTATC	GGCGTCACACCCCTCTATATT	BE405260
Xfcp414	CGTAGCGCGTCAATTATG	AGAACACCGAATGGAACGTC	BE405260
Xfcp404	CGTAGTGATCGAATTCTG	TGAGCATTGATCCTGAGTGG	BE405260
Xfcp410	GCGCGATGATAAATTATC	ACTGGGCATCCCACTCTACC	BE405260
Xfcp407	GTCGTACGTAGAATTCCT	TCGGATGAACATAGCCAGTG	BE497566
Xfcp406	GCGCGATGATAAATTATC	CCCATTCCTGTCGAGTTTGT	BE490440
Xfcp411	CGTAGCGCGTCAATTATG	GTGCCGCCTACATATCTGGT	BF474579
Xfcp418	CGTAGCGCGTCAATTATG	AGCGCCTTACTGACCGACTA	BF474778
Xfcp397	CGTAGTGATCGAATTCTG	CGTGTTGTTCGTGAAATTGG	BG604577
Xfcp401	CGTAGTGATCGAATTCTG	GACAAAGAGCCGCTGAAGTC	BE490304
Xfcp403	CGTAGTGATCGAATTCTG	CCCATTTGCCATTTATCACC	BE498683
Xfcp415	GGAACCAAACACATGAAGA	ACGCCACTGACCTTACTGCT	BG604977
Xfcp400	GGAACCAAACACATGAAGA	TTGCCGCGGTGTCACTTG	B15G03
Xfcp416	TTCTAGGTAATCCAACAACA	GGGGTTCTAAACAAGGTG	A11D14
Xfcp412	TTCTAGGTAATCCAACAACA	CGTAGTGAGGTAGTGACG	A11D14

* The GenBank accession number. Accession with a prefix of BJ, BG, BF or BE were the wheat ESTs mapped in the chromosome bin 3AS 0.45–1.00, while others were sunflower ESTs

The new molecular marker loci detected in this study, including STS and TRAP, were given *Xfcp* designations in accordance with the reserved laboratory designations of JD Faris (http://wheat.pw.usda.gov/ggpages/Lab.Designators.html).

Bulked segregant analysis

Bulked segregant analysis (BSA) was carried out to identify TRAP markers closely linked with *Qfhs.ndsu-3AS* as described by Haen et al (2004). DNA from eight RICLs with high FHB disease scores and a large portion of LDN chromosome 3A was combined as the susceptible bulk. DNA from eight RICLs with lower FHB disease scores and carrying a *T. dicoccoides* chromosomal fragment harboring the QTL were combined as the resistant bulk. The marker *Xfcp399* and *Xgwm666.1* flanking the QTL regions were used to form the resistant and susceptible bulks.

Linkage and QTL analysis

Mapmaker 2.0 (Lander et al. 1987) for Macintosh was used to detect linkage and calculate map

distances using the Kosambi mapping function (Kosambi 1944) as described in Liu et al. (2005). Because the RICLs underwent a single round of meiosis and recombinant chromosomes were essentially doubled as described by Joppa (1993), we considered the population as doubled haploids for map construction.

The computer program MapManager QTXb20 (Manly et al. 2001) was used for interval mapping of *Qfhs.ndsu-3AS* using the mean phenotypic data from five seasons of evaluation [three from the previous study (Otto et al. 2002) and two from the present study]. To determine the critical LOD threshold, we executed a permutation test with 1,000 permutations. A LOD threshold of about 2.0 in this RICL population yielded an experiment-wise significance level of 0.05.

Results

Phenotypic analysis of the mapping population

The population of 83 RICLs, LDN, and LDN-DIC 3A were evaluated for Type II FHB resistance over three seasons in a previous study (Otto et al. 2002). Reactions of these 83 RICLs and the parents to FHB were re-evaluated for two additional seasons in this study. A wide range of variation in FHB severity was observed in these two seasons as in the previous evaluations (Table 3). The frequency of the FHB severity averaged over the five seasons showed a normal distribution (Fig. 1). ANOVA indicated that there was a significant season effect on FHB severity across the five seasons. We eliminated season effects from the original data and generated the residual data without season effects via the regression analysis of FHB severity with seasons. The residual data derived from the regression analysis showed significant correlations among the five seasons, with correlation coefficients greater than the critical value of 0.217 ($P = 0.05$). Thus, we combined the residual data from five seasons for QTL analysis.

Molecular markers and saturation mapping

A number of molecular marker loci have been identified and mapped on chromosome 3A (Song et al. 2005; http://www.scabusa.org/pdfs/BARC_SSRs_011101.html; Somers et al. 2004; http://wheat.pw.usda.gov/ggpages/SSR/WMC/WMCPri13.xls). We surveyed eight BARC and ten WMC SSR primer sets for polymorphisms between the parents and between the resistant and susceptible bulks. Four pairs of BARC primers and three pairs of WMC primers detected polymorphisms, but only three BARC SSR loci (*Xbarc19*, *Xbarc45* and *Xbarc51*) mapped to chromosome 3A. *Xbarc45* mapped 3.6 cM distal to *Xgwm2*, an SSR marker locus closely linked to the QTL peak (Otto et al. 2002) (Fig. 2).

Qfhs.ndsu-3BS, a major FHB resistance QTL identified in common wheat, was mapped on the short arm of chromosome 3B (3BS). We screened LDN and LDN-DIC 3A for polymorphisms with 28 STS markers assigned to the chromosomal region harboring *Qfhs.ndsu-3BS* on 3BS (Liu and Anderson 2003b). Eight of them detected polymorphisms between LDN and LDN-DIC 3A and mapped distal to the QTL region on chromosome 3A. One STS detected an additional locus

Table 3. FHB severity in five greenhouse seasons

Individuals	Spring 1998*	Winter 1998	Spring 1999 *	Winter 2003	Spring 2005
Mean severity of RICLs	54.2 ± 18.3	24.8 ± 11.8	27.0 ± 13.19	18.4 ± 9.7	35.8 ± 11.7
Severity range of RICLs	18.0–93.3	5.6–68.5	8.2–59.6	3.9–46.2	16.5–60.3
Severity of LDN-DIC 3A	18.86	12.8	–	8.0 ± 1.1	19.3 ± 1.8
Severity of LDN	69.73	–	54.64	47.1 ± 2.0	66.9 ± 2.4
Severity of Sumai 3	–	–	–	6.2 ± 0.001	7.3 ± 0.002
Severity of LDN-DIC 2A	–	–	–	71.5 ± 2.6	77.7 ± 3.7

* Data from Otto et al. (2002)

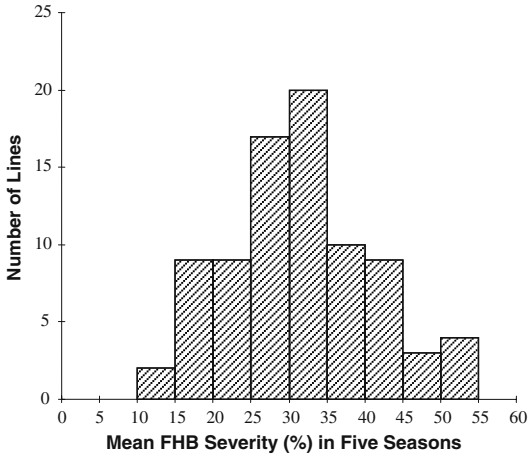

Fig. 1 Distribution of the mean FHB severity across the five seasons of evaluation

(*XSTS3B55.2*) proximal to the QTL region (Fig. 2).

A linkage map of chromosome 3A with 19 molecular marker loci spanning a genetic distance of 155.2 cM was constructed, including the SSR marker locus *Xgwm2* that defined the QTL peak in a previous study by Otto et al. (2002). We assigned *Xgwm2* to the chromosome bin 3AS 0.45–1.00 using Chinese Spring (CS) (*T. aestivum* L.) deletion lines of 3AS in 2003 (Chen and Cai, unpublished). Deletion mapping by Sourdille et al. (2004) also mapped *Xgwm2* to this chromosome bin. The ESTs mapped in this chromosome bin were used as reference sequences to identify STS, SSR, and TRAP marker loci within this chromosomal region. From 165 ESTs, we identified 13 that contained NBS and/or LRR domains or had significant similarity to kinase-like genes. One of the 13 EST-derived STS markers (*Xfcp408*) mapped to chromosome 3A. A search for microsatellites identified 17 ESTs that contained at least one SSR. Seventeen pairs of SSR primers were designed based on the DNA sequences flanking the SSRs, and nine detected polymorphisms between the parents. Unfortunately, none of them mapped to chromosome 3A. A total of 100 STS primer pairs were designed from the remaining ESTs within the chromosome bin. Fourteen of them amplified polymorphisms between the two parents, but only four STS loci

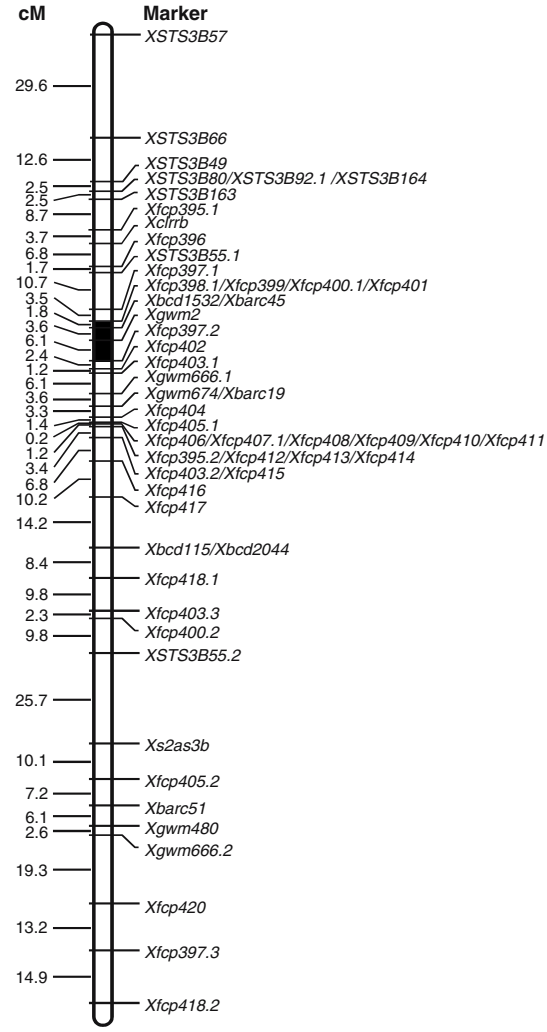

Fig. 2 Genetic linkage map of chromosome 3A generated in the LDN x LDN-DIC 3A RICL population. Marker loci are listed to the right and centiMorgan (cM) distances are shown to the left. The shaded region indicates the chromosomal interval harboring *Qfhs.ndsu-3AS*

(*Xfcp396*, *Xfcp399, Xfcp402*, and *Xfcp420*) were detected on chromosome 3A (Fig. 2).

We surveyed the parents and the bulks with 365 fixed/random primer combinations in an effort to identify TRAP loci closely linked to the QTL *Qfhs.ndsu-3AS*. Seventy-six (~21%) of them detected polymorphisms and their segregation in the mapping population was analyzed, but only 22 TRAP loci were detected on chromosome 3A. One of the TRAP markers, *Xfcp397.2*, mapped 6.1 cM proximal to *Xgwm2* (Fig. 2).

Some of the TRAP primer pairs detected duplicate loci on chromosome 3A, such as *Xfcp397.1* and *Xfcp397.2* (Fig. 2), or on different chromosomes, including chromosome 3A. The TRAP primer pair that detected the loci *Xfcp418.1* and *Xfcp418.2* on chromosome 3A amplified an additional polymorphic fragment not locating on chromosome 3A (Figs. 2 and 3).

A total of 55 marker loci, including SSR, STS, TRAP, and RFLP, were assigned to chromosome 3A resulting in a linkage map spanning a genetic distance of 277.2 cM (Fig. 2). The average marker density of this map was one marker per 5.1 cM.

QTL analysis

Composite interval mapping was performed using combined residual data from five seasons and the linkage map constructed in this study (Fig. 2). The SSR marker *Xgwm2* was the most significant ($P < 0.00001$) in single marker regression and explained 38% of the phenotypic variation for resistance to FHB. Composite interval mapping indicated that *Qfhs.ndsu-3AS* explained 42% of the phenotypic variation for FHB resistance (Fig. 4). *Qfhs.ndsu-3AS* was positioned within a chromosomal interval of 11.5 cM via graphical genotyping (Fig. 5). This QTL is flanked by the TRAP marker *Xfcp401* and *Xfcp397.2*. Three other markers, *Xfcp398.1*, *Xfcp399*, and *Xfcp400.1*, cosegregated with *Xfcp401* in the RICL population (Fig. 2). Two cosegregating markers, *Xbcd1532* and *Xbarc45*, and *Xgwm2* resided within the QTL region (Figs. 2 and 5).

Comparative mapping of the chromosomal regions harboring *Qfhs.ndsu-3AS* and *Qfhs.ndsu-3BS*

We used 28 STS markers assigned to the chromosomal deletion bin 3BS 0.78–0.87 containing *Qfhs.ndsu-3BS* (Liu and Anderson 2003b) to detect the colinear region harboring these STS marker loci on chromosome 3A. Eight of them were mapped on 3A, but all of them were localized outside of the chromosomal region spanning *Qfhs.ndsu-3AS* (Figs. 2 and 4). A fine map of *Qfhs.ndsu-3BS* recently developed by Liu et al. (2006) placed this QTL within a chromosomal interval of 1.2 cM flanked by the STS marker *XSTS3B-189* and *XSTS3B-206*. The STS marker *XSTS3B-80* between these two flanking markers mapped 37.6 cM distal to the chromosomal region harboring *Qfhs.ndsu-3AS*. The STS marker *XSTS3B-66*, which mapped proximal to the QTL region on 3BS, mapped distal to *XSTS3B-80* on 3AS (Figs. 2 and 5). In addition, two RFLP marker loci (*Xfba91-3A* and *Xcdo1164-3A*) distal to the markers mapped within and proximal to the QTL region on 3AS were found to reside far proximal to the QTL region on 3BS (*Xfba91-3B* and *Xcdo1164-3B*) (Fig. 5). On the other hand, two RFLP marker loci (*Xtam47-3B* and *Xtam61-3B*) proximal to the marker loci flanking the QTL region on 3BS were found to reside far distal to the QTL region on 3AS (*Xtam47-3A* and *Xtam61-3A*) (Fig. 5). Therefore, *Qfhs.ndsu-3AS* and *Qfhs.ndsu-3BS* are not likely to be homoeoloci (Fig 6).

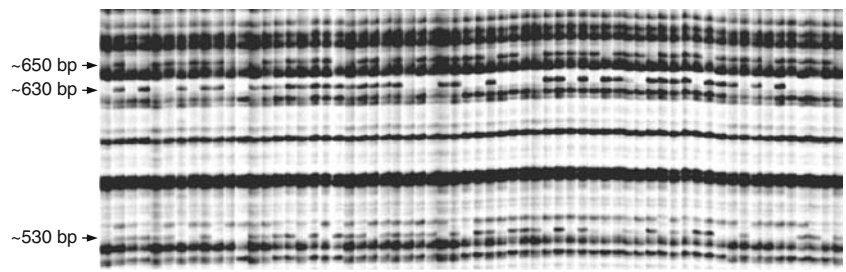

Fig. 3 A polyacrylamide gel showing three TRAP fragments amplified by one primer pair in LDN (lane 1 from left), LDN-DIC 3A (lane 2 from left), and 61 RICLs (lanes 3 through 63 from left to right). Arrows point to the three polymorphic fragments. The middle (~630 bp) and bottom (~530 bp) fragments were mapped to chromosome 3A (*Xfcp418.1* and *Xfcp418.2*). The top fragment (~650 bp) was mapped to another chromosome rather than 3A

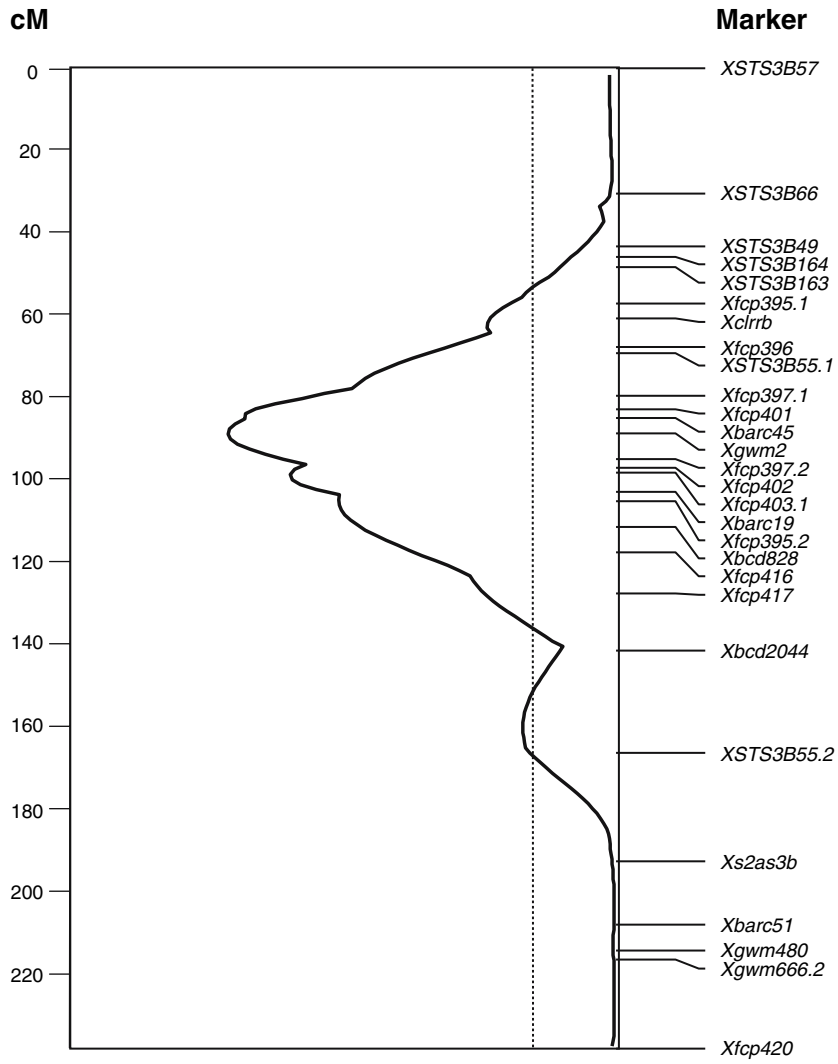

Fig. 4 Interval regression mapping of *Qfhs.ndsu-3AS* in the RICL population derived from LND x LDN-DIC 3A. A centiMorgan (cM) scale is indicated to the left, and markers are indicated to the right. The dotted line represents the LOD threshold of 2.0

Discussion

To date, *Qfhs.ndsu-3AS* is the only FHB resistance QTL identified in tetraploid wheat. A linkage map of chromosome 3A with 19 molecular marker loci spanning a genetic distance of 155.2 cM was constructed in a previous study by Otto et al. (2002). In the present study, we saturated this genetic map with 45 new molecular markers and constructed a linkage map of chromosome 3A with 55 marker loci spanning a genetic distance of 277.2 cM. The marker density of this map was increased from one marker per 8.6 cM to 5.1 cM. About 10 new markers detected loci near *Xgwm2*. The increase in marker density in the chromosomal region harboring *Qfhs.ndsu-3AS* led to a better resolution of recombination breakpoints within this region and allowed positioning of the QTL within a smaller chromosomal interval (11.5 cM) compared to the previous study (Otto et al. 2002).

The vast EST pool in wheat is an invaluable resource for genome mapping (Qi et al. 2004). We designed a total of 514 pairs of TRAP, STS, and SSR primers based on the ESTs assigned to the chromosomal deletion bin 3AS 0.45–1.00 (http://wheat.pw.usda.gov/cgi-bin/westsql/map_locus.cgi). Thirty-three EST-derived marker loci, including TRAP, STS, and SSR, were identified on chromosome 3A. However, only one of them,

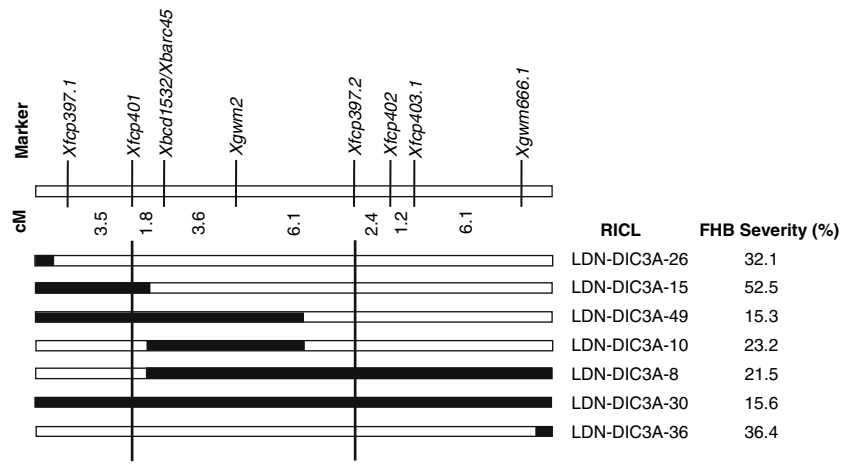

Fig. 5 Graphical genotypes of seven RICLs. The open and black portions of the bar represent alleles from LDN and *T. dicoccoides*, respectively. The recombination points between two marker loci were assumed to be in the middle of the chromosomal region between them. *Qfhs.ndsu-3AS* was placed within an 11.5 cM interval flanked by *Xfcp401* and *Xfcp397.2* (between two vertical lines) based on the genotypes of the RICLs at the marker loci and their FHB severity

Xfcp397.2, resided within the QTL region and mapped 6.1 cM proximal to *Xgwm2*. These results suggested that this chromosomal interval spanning the QTL *Qfhs.ndsu-3AS* might not be a gene-rich region. Previous evidence suggests that ESTs within 3AS 0.45–1.00 tend to be clustered in a small chromosomal interval (Munkvold et al. 2004). 3AS was reported to be one of the arms having a significantly lower gene density than expected (Qi et al. 2004). Another chromosomal interval of 2.8 cM flanked by the two TRAP loci *Xfcp404* and *Xfcp414* harbored eleven EST-derived TRAP markers, one EST-derived STS marker, and one RGA-derived STS marker on chromosome 3A. This chromosomal interval may be a gene-rich region, or recombination may be suppressed within this interval.

Some of the fixed primers derived from the same EST amplified multiple TRAP loci on chromosome 3A in combinations with different random primers, such as *Xfcp405*, *Xfcp414*, *Xfcp404*, and *Xfcp410*. This might result from the use of a low annealing temperature in the TRAP PCR (Hu and Vick 2003). This molecular marker technique, however, has proven repeatable and reliable in plants (Hu and Vick 2003; Liu et al. 2005; Yang et al. 2005).

Segregation at 114 marker loci was analyzed in the mapping population of 83 RICLs. Unfortunately, only 45 of them were mapped to chromosome 3A and many were assigned to other chromosomes. This may have resulted from the genomic differences between LDN and LDN-DIC 3A in addition to chromosome 3A. The LDN-DIC substitution lines, including LDN-DIC 3A, were developed from the crosses of LDN-D genome substitution lines (Joppa 1993) with the *T. dicoccoides* accession Israel-A. The initial substitution lines were backcrossed to a corresponding LDN-D genome substitution line five times in order to recover the LDN genomic background except for the chromosome substituted by the homologous *T. dicoccoides* chromosome (Williams and Joppa 1988). A small portion of the *T. dicoccoides* genomes might have remained in the LDN-DIC substitution lines in addition to chromosome 3A from *T. dicoccoides* because only five backcrosses were conducted. LDN D-genome substitution lines were derived from the crosses of LDN with CS nulli-tetrasomic lines. The substitution lines selected from the progeny of these crosses were backcrossed to LDN seven times to recover the LDN genomic background except the chromosome replaced by a D-genome chromosome (Joppa 1993). Again, a small percentage of the CS genomes could have remained in the LDN-D genome substitution lines. Existence of the partial *T. dicoccoides* and

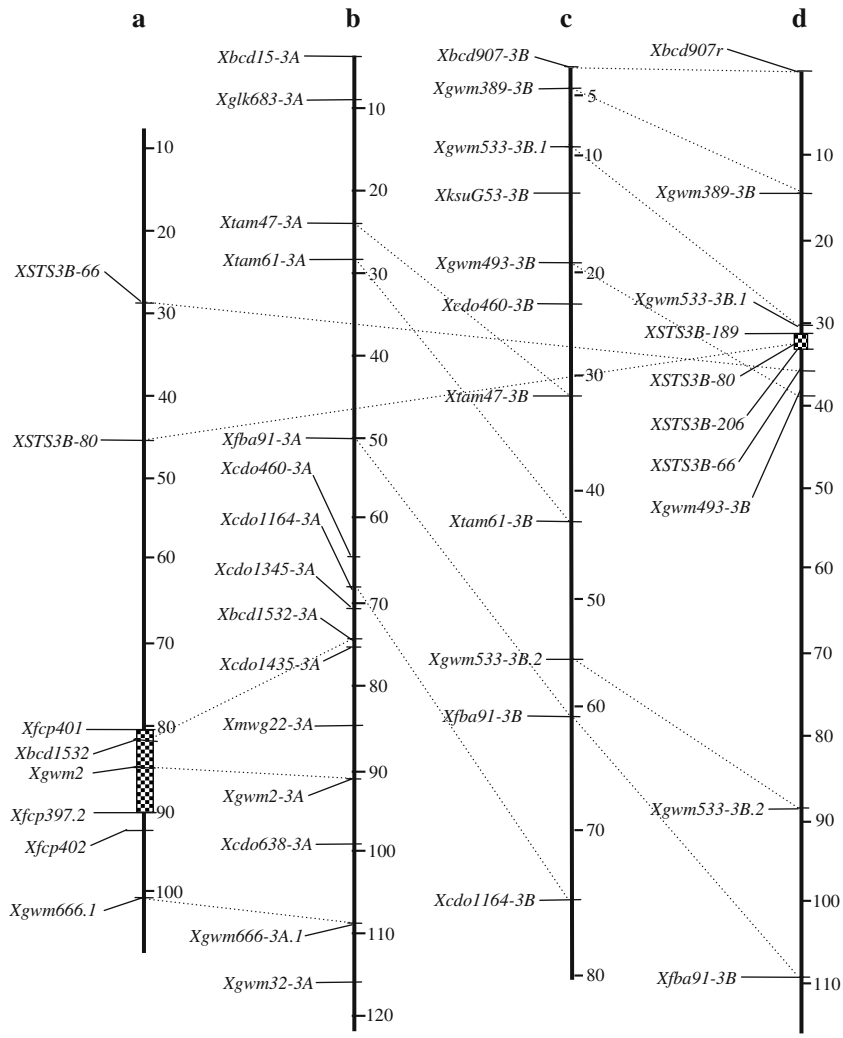

Fig. 6 Comparative maps of the chromosomal regions harboring *Qfhs.ndsu-3BS* and *Qfhs.ndsu-3AS*. (**a**) Partial genetic map of the chromosomal region harboring *Qfhs.ndsu-3AS*; (**b**) CMap of 3AS (http://wheat.pw.usda.gov/GG2/index.shtml); (**c**) Partial CMap of 3BS (http://wheat.pw.usda.gov/GG2/index.shtml); (**d**) Partial fine map of *Qfhs.ndsu-3BS* (Liu and Anderson 2003b; Liu et al. 2006). The patterned regions indicate the chromosomal intervals harboring *Qfhs.ndsu-3BS* and *Qfhs.ndsu-3AS*, respectively. Marker loci are listed to the left and centiMorgan (cM) distances are shown to the right.

CS genomes in the LDN-DIC 3A and LDN-D genome substitution line 3D(3A) could have led to allelic variations at loci on chromosomes other than 3A in LDN and LDN-DIC 3A. This anomaly was also found in a previous study in which the LDN-DIC RICLs of chromosomes 6A and 6B were involved (Du and Hart 1998).

A high degree of colinearity exists between the homoeologous chromosomes 3A and 3B in wheat. The FHB resistance QTL *Qfhs.ndsu-3BS* and *Qfhs.ndsu-3AS* were localized on the short arm of the chromosome 3B and 3A, respectively (Waldron et al. 1999; Otto et al. 2002). Comparative mapping of the chromosomal regions harboring the QTL *Qfhs.ndsu-3BS* and *Qfhs.ndsu-3AS* suggested that these two QTL were not homoeologous.

Fusarium head blight resistance has a complex inheritance pattern and evaluation of FHB resistance is a time and labor consuming work. Identification of molecular marker loci closely linked to the FHB resistance QTL could facilitate selection of this complex trait in breeding. Eight PCR-based molecular markers, including STS, SSR, and TRAP, were mapped in the chromosomal region containing the QTL *Qfhs.ndsu-3AS*. These PCR-based markers could be utilized to assist selection of *Qfhs.ndsu-3AS* in breeding materials and to enhance utilization of this QTL in the development of durum varieties resistant to

FHB. In addition, *Qfhs.ndsu-3AS* was transferred to the hexaploid wheat background and has been utilized in breeding for FHB resistance in common wheat (Hartel et al. 2004). The resistant RICLs that contain the smallest *T. dicoccoides* fragments harboring *Qfhs.ndsu-3AS* will be useful for the introgression of this resistance QTL to adapted wheat backgrounds. Molecular markers generated in this study could facilitate pyramiding of this QTL and other FHB resistance QTL identified in common wheat.

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