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ORIGINAL ARTICLE

Toward a better understanding of the genomic region harboring Fusarium head blight resistance QTL *Qfhs.ndsu-3AS* in durum wheat

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Abstract

Key message **New molecular markers were developed and mapped to the FHB resistance QTL region in high resolution. Micro-collinearity of the QTL region with rice and *Brachypodium* was revealed for a better understanding of the genomic region.**

Abstract The wild emmer wheat (*Triticum dicoccoides*)-derived Fusarium head blight (FHB) resistance quantitative trait locus (QTL) *Qfhs.ndsu-3AS* previously mapped to the short arm of chromosome 3A (3AS) in a population of recombinant inbred chromosome lines (RICLs). This study aimed to attain a better understanding of the genomic region harboring *Qfhs.ndsu-3AS* and to improve the utility of the QTL in wheat breeding. Micro-collinearity of the QTL region with rice chromosome 1 and *Brachypodium* chromosome 2 was identified and used for marker development in saturation mapping. A total of 42 new EST-derived sequence tagged site (STS) and simple sequence repeat (SSR) markers were developed and mapped to the QTL and nearby regions on 3AS. Further comparative analysis revealed a complex collinearity of the 3AS genomic region with their collinear counterparts of rice and *Brachypodium*. Fine mapping of the QTL region resolved five co-segregating markers (*Xwgc1186*/*Xwgc716*/*Xwgc1143*/*Xwgc501*/*Xwgc1204*) into three distinct loci proximal to *Xgwm2*, a marker previously reported to be closely linked to the QTL. Four other markers (*Xwgc1226*, *Xwgc510*, *Xwgc1296*, and *Xwgc1301*) mapped farther proximal to the above markers in the QTL region with a higher resolution. Five homozygous recombinants with shortened *T. dicoccoides* chromosomal segments in the QTL region were recovered by molecular marker analysis and evaluated for FHB resistance. *Qfhs.ndsu-3AS* was positioned to a 5.2 cM interval flanked by the marker *Xwgc501* and *Xwgc510*. The recombinants containing *Qfhs.ndsu-3AS* and new markers defining the QTL will facilitate utilization of this resistance source in wheat breeding.

Introduction

Fusarium head blight (FHB), caused mostly by *Fusarium graminearum* Schwabe [telomorph: *Gibberella zeae* (Schw.) Petch] in North America, is a destructive fungal disease of wheat. Infection and development of FHB are accelerated by warm and humid conditions (Stack and McMullen 1985; Xu et al. 2008). Outbreak of FHB can cause severe losses of grain yield and quality in both common wheat (*Triticum aestivum* L., $2n = 6x = 42$, genome AABBDD) and durum wheat (*T. durum* Desf., $2n = 4x = 28$, genome AABB) (McMullen et al. 1997). Multiple QTL for FHB resistance have been identified and successfully deployed in common wheat cultivars (Liu and Anderson 2003; Liu et al. 2006, 2008; Buerstmayr et al.

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2009; Chu et al. 2011). However, FHB remains a major threat to durum wheat production due to the lack of effective resistance sources in durum (Oliver et al. 2007). Incorporation of FHB resistance genes from common wheat into durum has not been very successful because of the complex inheritance of hexaploid-derived FHB resistance in the durum background (Buerstmayr et al. 2012; Zhu et al. 2012).

Sources of FHB resistance have been found in durum wheat and its tetraploid relatives even though they are not as effective as those in common wheat (Stack et al. 2002; Buerstmayr et al. 2003; Cai et al. 2005; Oliver et al. 2007, 2008; Ghavami et al. 2011; Talas et al. 2011; Ruan et al. 2012; Zhang et al. 2014). Molecular mapping has identified several wild emmer wheat (*T. dicoccoides*)-derived FHB resistance QTL, including *Qfhs.ndsu-3AS* on chromosome 3A (Otto et al. 2002), *Qfhs.fcu-7AL* on 7A (Kumar et al. 2007), and another on 6B (Stack et al. 2006). Also, FHB resistance QTL has been detected in the durum-related tetraploids *T. cathlicum* (Somers et al. 2006) and *T. dicoccum* (Buerstmayr et al. 2012; Zhang et al. 2014). In addition, extensive screening of durum accessions for FHB resistance has identified several durum landraces with detectable resistance to the disease over the last few years (Ghavami et al. 2011; Talas et al. 2011; Zhang et al. 2014). Some of these tetraploid-derived FHB resistance QTL mapped to the same genomic regions as those identified in hexaploids, suggesting collinearity of the resistance gene loci in tetraploids and hexaploids (Somers et al. 2006; Buerstmayr et al. 2012).

Qfhs.ndsu-3AS is a wild emmer-derived FHB resistance QTL located on 3AS (Otto et al. 2002; Stack et al. 2002). It is the first FHB resistance QTL identified in tetraploid wheat and confers moderate resistance in the durum background. Saturation mapping positioned *Qfhs.ndsu-3AS* to a chromosomal interval of 11.5 cM flanked by the molecular markers *Xfcp401* and *Xfcp397.2* on 3AS (Chen et al. 2007). Wheat chromosomes in homoeologous group 3 are collinear to rice chromosome 1 (R1) and *Brachypodium* chromosome 2 (B2) (Moore et al. 1995; the International Brachypodium Initiative 2010; Sehgal et al. 2012; Luo et al. 2013). Wheat researchers have been taking advantage of the collinearity with these two grass models to characterize genes in the large and complex polyploid genome of wheat (Liu and Anderson 2003; Foote et al. 2004; Liu et al. 2006; Chen et al. 2007; Kumar et al. 2009). This study aimed to saturate the genomic region harboring *Qfhs.ndsu-3AS* and improve the map resolution of the QTL region by exploring the micro-collinearity of the QTL region with R1 and B2. This will facilitate understanding of the genomic region harboring the FHB resistance QTL *Qfhs.ndsu-3AS* and provide effective molecular markers to assist selection of this resistance QTL in wheat breeding.

Materials and methods

Mapping populations

Eighty-three recombinant inbred chromosome lines (RICLs) derived from the cross between durum wheat cultivar 'Langdon' (LDN) and disomic LDN-*T. dicoccoides* Israel-A (ISA) substitution line 3A [LDN(DIC-3A)] (Joppa 1993) were employed for saturation mapping and QTL analysis in this study. RICL#10, having the shortest *T. dicoccoides* chromosomal fragment harboring *Qfhs.ndsu-3AS*, was crossed with LDN to generate additional meiotic recombinants within the QTL region for fine mapping of the QTL. A large F_2 population ($n > 1800$) segregating only in the QTL region was developed from that cross. The F_2 individuals with *T. dicoccoides* chromosomal fragments smaller than that in RICL#10 were selected to develop recombinants homozygous at the marker loci within the QTL region. Two other RICLs (RICL#15 and RICL#49) critical in the QTL region were also included in this study. Both RICL#10 and RICL#49 exhibited FHB resistance as the resistant parent LDN(DIC-3A), while RICL#15 exhibited susceptibility to FHB as the susceptible parent LDN (Chen et al. 2007). RICL#10 and the homozygous recombinants developed in this study were evaluated for FHB resistance to identify shortened *T. dicoccoides* chromosomal fragments harboring *Qfhs.ndsu-3AS* for fine mapping of the QTL and for germplasm development.

Comparative analysis and molecular marker development

An initial comparative analysis was performed to develop EST (expressed sequence tag)-derived STS and SSR markers for saturation mapping of the *Qfhs.ndsu-3AS* QTL region based on the collinearity of the QTL region with the genomic regions on rice chromosome R1. The EST sequences of BE517736 and BF484475, from which the STS markers *Xfcp402* and *Xfcp399* flanking the QTL region were developed (Chen et al. 2007), were used as queries to perform BLASTn against rice and *Brachypodium* genomic sequences and to identify the collinear counterparts of the QTL region on rice chromosome R1 and *Brachypodium* chromosome B2 using the J. Craig Venter Institute (JCVI) wheat genome database (http://blast.jcvi.org/euk-blast/index.cgi?project=tae1) and *Brachypodium* database (http://www.brachypodium.org/gmod/alignment/blast_finders/new), respectively. A threshold of an expected (E) value equal to or less than e^{-15} was adopted in the BLAST search and comparative analysis. The rice bacterial artificial chromosome (BAC)/P1-derived artificial chromosome (PAC) clones and *Brachypodium* genomic sequence hits in the BLAST search were used as anchor

points to identify the rice and *Brachypodium* genomic regions collinear to the QTL region. Then the retrieved rice and *Brachypodium* genomic sequences were used as queries to search for wheat ESTs in the JCVI wheat genome database. The corresponding tentative sequences (TCs) (http://compbio.dfci.harvard.edu/cgi-bin/tgi/est_ann.pl?gudb=wheat) or contigs in GrainGenes 2.0 (http://wheat.pw.usda.gov/GG2/index.shtml) for the identified ESTs were adopted to eliminate redundant EST sequences. The STS and SSR primers were designed from the EST sequences using Primer 3 (http://biotools.umassmed.edu/bioapps/primer3_www.cgi).

Genetic mapping and QTL analysis

All EST-derived STS and SSR primers were surveyed for polymorphisms between LDN and LDN (DIC-3A). The polymorphic markers were employed to genotype the RICL population ($n = 83$) for saturation mapping of the FHB resistance QTL *Qfhs.ndsu-3AS*. The markers mapped to the QTL region were used to genotype a subset of the F_2 population ($n = 372$) for fine mapping of the QTL. PCR amplification was carried out in a 20-µl mixture containing 40 ng genomic DNA, 0.5 µM each of forward and reverse primers, 1× PCR buffer, 1.5 mM $MgCl_2$, 0.25 mM dNTP and 0.25 U of *Taq* DNA polymerase. PCR was run as follows: 94 °C for 3 min; 45 cycles of 94 °C for 1 min, 52~60 °C (varied with specific primer pairs) for 1 min and 72 °C for 1.5 min; then with a final 72 °C for 7 min. PCR products were separated on 8 % non-denaturing polyacrylamide gel and visualized by ethidium bromide staining or separated on 5 % denaturing polyacrylamide gel and visualized by silver staining (Liu and Anderson 2003; Chen et al. 2007). Additional molecular marker techniques used in this study included cleaved amplified polymorphism (CAP) (Chen et al. 2003), single-strand conformation polymorphism (SSCP) (Kumar et al. 2006), and heteroduplex analysis (Mohamed et al. 2004). The genetic maps were constructed using Mapmaker 2.0 for Macintosh as described by Lander et al. (1987). All the newly developed molecular markers in this project were designated *Xwgc* followed by a number.

Qfhs.ndsu-3AS was analyzed against the saturated linkage map of the QTL region in the RICL population using the computer software QGene 4.3.10 (http://www.qgene.org/qgene/index.php). The same sets of phenotypic data as those used in the previous study by Chen et al. (2007) were employed in the composite interval mapping. A permutation test with 1000 permutations was performed to determine the LOD threshold. A LOD threshold of about 2.0 was determined with an experiment-wise error rate of 0.05 in the RICL population.

Immature embryo culture

The immature embryo culture technique was used to accelerate generations of the recombinants for FHB evaluation. Immature embryos of the recombinants at early generations were excised from caryopses and cultured in vitro on MS medium (Cai 1993). Seedlings regenerated from immature embryos were transplanted to the greenhouse for further studies.

FHB disease evaluation and data analysis

The recombinants homozygous at the molecular marker loci within the QTL region were recovered from the LDN x RICL#10 cross by molecular marker analysis. They were grown for evaluation of FHB resistance in randomly arranged 6-inch plastic pots with one plant per pot in 2–3 greenhouse seasons (Fall 2013, Spring 2013, and Summer 2014). Their parents, RICL#10 and LDN, were used as resistant and susceptible controls, respectively. Response of the recombinants and their parents to FHB was evaluated by the point inoculation method as described by Stack et al. (2002). A single floret from a central spikelet was inoculated at first anthesis using 10 µl of spore suspension. The inoculum was prepared by mixing equal number of spores harvested from four isolates of *F. graminearum* and concentrated to 1×10^5 spores per mL. The temperature in the greenhouse was kept at approximately 25 °C with a 16 h photoperiod during the disease development stage. To facilitate disease development, high humidity was maintained for 72 h with the inoculated spikes covered by a plastic bag. At 21 days post inoculation, the percentage of infected spikelets in a spike was scored. For each pot, the average percentage of all spikes (5–10) was calculated as FHB severity for each individual homozygous recombinant. With regard to the control, the mean value of three pots was recorded as FHB severity.

ANOVA was performed on FHB severity of the homozygous recombinants and their parents. Fisher's protected LSD was used for mean separation between the genotypes. All statistical analyses were conducted using the Statistical Analysis System version 9.4.

Results

Identification and analysis of micro-collinearity

The wheat ESTs BE517736 (*Xfcp402*) and BF484475 (*Xfcp399*) mapped proximally and distally to the *Qfhs.ndsu-3AS* QTL region defined in the previous study by Chen et al. (2007). The contig sequences of BE517736 (Ta.9622.1.S1_x_at) and BF484475 (Ta.1999.3.S1_a_at)

were used as queries of BLAST search against rice genomic sequences. One rice PAC (AP003282) and one rice BAC (AP004225) were hit by the contig 'Ta.9622.1.S1_x_at' with the lowest E values of $3.2e^{-144}$ and $6.8e^{-90}$, respectively. AP003282 is located on the short arm of rice chromosome 1, while AP004225 is on the long arm. Since *Qfhs.ndsu-3AS* mapped to the short arm of wheat chromosome 3A (3AS), AP003282 was selected as the anchor point of BE517736 on rice chromosome 1. BLAST search with the contig 'Ta.1999.3.S1_a_at' identified three PACs (AP003610, AP002969, and AP003727) on rice chromosome 1 with similar E values, i.e., $5.4e^{-40}$, $5.8e^{-40}$, and $8.0e^{-40}$, respectively. Sequence alignment of the three PACs indicated that AP003610 was part of AP003727 and 84 % of AP002969 was included in AP003727. Thus, AP003727 was considered the anchor point of BF484475 on rice chromosome 1. Therefore, the rice genomic region from AP003727 to AP003282 (3724 kb) was considered collinear with the wheat genomic region spanning *Qfhs.ndsu-3AS* on 3AS. BLAST search against the *Brachypodium* genome with BE517736 identified a genomic region from 3139,341 to 3141,007 bp on chromosome 2 with an E value of $1.4e^{-131}$, while BF484475 identified a genomic region from 2,644,263 to 2,644,440 bp with an E value of $4.7e^{-53}$ on the same chromosome. Thus, the *Brachypodium* genomic region from 2,644,263 to 3,141,007 bp on chromosome 2 was considered collinear with the wheat genomic region spanning *Qfhs.ndsu-3AS* on 3AS.

Molecular mapping and QTL analysis

A total of 793 pairs of STS primers and 42 pairs of SSR primers were designed from the wheat EST singletons, TCs, and contigs identified based on the micro-collinearity of the *Qfhs.ndsu-3AS* QTL region with the genomic regions of rice and *Brachypodium*. Forty-eight pairs of STS primers and three pairs of SSR primers amplified polymorphisms between the two parents of the mapping population. Of these, 42 new STS/SSR markers mapped to a genomic region of 143.3 cM spanning the QTL on 3AS (Fig. 1). The primer sequences of the newly mapped STS and SSR markers on 3AS are listed in Table 1. Twenty-two of the newly developed markers mapped within the QTL region previously defined by the marker *Xfcp401* and *Xfcp397.2* (Chen et al. 2007). Five co-segregating STS markers, *Xwgc501*, *Xwgc716*, *Xwgc1143*, *Xwgc1188*, and *Xwgc1204*, mapped 0.6 cM proximal to *Xgwm2*, an SSR marker locus identified closely linked to the QTL peak in previous studies (Otto et al. 2002; Chen et al. 2007). Two co-segregating markers (*Xwgc774*/*Xwgc1226*) mapped 1.2 cM proximal to the five co-segregating markers within the QTL region. Immediately proximal to *Xwgc774*/*Xwgc1226* were three other co-segregating markers (*Xwgc510*, *Xwgc1296*, and *Xwgc1301*) (Fig. 1). In addition, twelve markers mapped closely distal to the *Xbcd1532*/*Xbarc45* loci. Ten of them, including *Xwgc1238*, *Xwgc1240*, *Xwgc1248*, *Xwgc1241*, *Xwgc1244*, *Xwgc901*, *Xwgc1263*, *Xwgc1254*, *Xwgc1100*, and *Xwgc1094*, co-segregated in the RICL mapping population. In addition, 11 newly developed markers mapped distal to the previously defined QTL region (i.e., distal to *Xfcp401*) (Chen et al. 2007; Fig. 1). The distal region defined by these eleven markers on 3AS is collinear with the genomic region encompassing the *Fhb1* locus on the short arm of wheat chromosome 3B (Chen et al. 2007; Fig. 1).

Composite interval mapping of *Qfhs.ndsu-3AS* against the saturated genetic map delimited the QTL to the chromosomal segment of 6.2 cM flanked by *Xbarc45* and *Xwgc510* in the RICL population (Figs. 1, 2). Each of the four intervals defined by the markers within the chromosomal segment (i.e., *Xbarc45-Xgwm2*, *Xgwm2-Xwgc501*, *Xwgc501-Xwgc1226*, and *Xwgc1226-Xwgc510*) explained a phenotypic variation ranging from 43.3 to 46.9 % with a LOD score of over 10.0, which were higher than the intervals outside the chromosomal segment flanked by *Xbarc45* and *Xwgc510* (Fig. 2).

Nine co-dominant STS markers (*Xwgc1188, Xwgc716, Xwgc1143, Xwgc501, Xwgc1204, Xwgc1226, Xwgc1296, Xwgc1301*, and *Xwgc510*) and two SSR markers (*Xbarc45* and *Xgwm2*) mapped to the QTL region (Fig. 1) were used to genotype the F_2 mapping population ($n = 372$). *Xwgc774*, co-segregating with *Xwgc1226*, was not included because it is a dominant marker and could affect mapping accuracy in an F_2 population (Jiang and Zeng 1997). *Xbarc45*, a dominant SSR marker, was used to identify homozygous recombinants, but was not used in fine mapping. *Xwgc716* and *Xwgc1188* still co-segregated and mapped 0.8 cM proximal to *Xgwm2* in the F_2 population. *Xwgc1143* and *Xwgc1204* also co-segregated and mapped 0.1 cM proximal to *Xwgc716*/*Xwgc1188* (Fig. 3). Five other STS markers, including *Xwgc501*, *Xwgc1226*, *Xwgc510*, *Xwgc1296*, and *Xwgc1301*, mapped farther proximal to the above markers. The map distances from *Xwgc1226* to its flanking markers *Xwgc501* (distal) and *Xwgc510* (proximal) were stretched, whereas *Xwgc1296* and *Xwgc1301* still co-segregated in the F_2 population (Figs. 1, 3).

Identification and evaluation of recombinants for FHB resistance

Genotyping of the F_2 population ($n = 372$) at the 11 SSR and STS marker loci within the QTL region identified three meiotic recombinants (3AS07-39-17, 3AS07-42-26, and 3AS07-53-16). They all contained a shortened *T. dicoccoides* 3AS fragment in the QTL region (Table 2). 3AS07-39-17 was homozygous at all 11 marker loci in the QTL region and designated 3AS-Rec I (Table 2). Prior to FHB

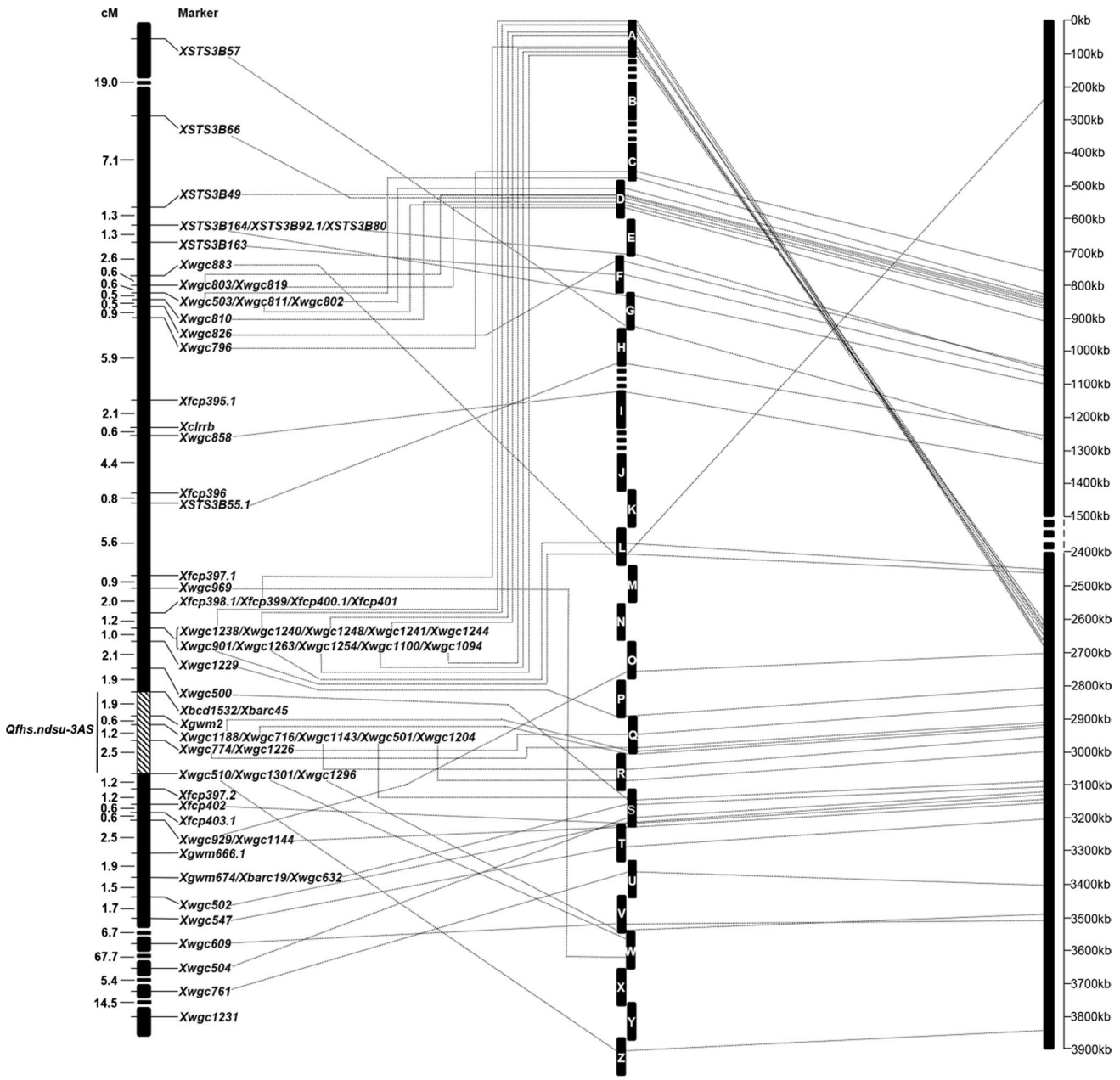

Fig. 1 Saturated genetic map of wheat 3AS distal region constructed in the RICL population (*left*), physical map of the distal region (4360 kb) of rice R1S (*middle*), and distal region of *Brachypodium* B2S (*right*) and comparative analysis of these three genomic regions. The *hatched region* refers to the QTL region in the genetic map (*left*). The *breaks* refer to the regions where genetic and physical map distances are not to scale. Rice PACs/BACs: *A* AP003727, *B* AP002882, *C* AP002747, *D* AP002541, *E* AP002868, *F* AP002487, *G* AP003046, *H* AP003233, *I* AP002538, *J* AP002872, *K* AP002540, *L* AP002522, *M* AP003045, *N* AP003225, *O* AP002521, *P* AP003209, *Q* AP003301, *R* AP003339, *S* AP003282, *T* AP003215, *U* AP002523, *V* AP002903, *W* AP002524, *X* AP003118, *Y* AP003047, *Z* AP002484

evaluation, this recombinant line was advanced to the F_3 and F_4 generations to increase homozygosity at other loci. 3AS07-42-26 and 3AS07-53-16 were heterozygous at some of the marker loci within the QTL region (Table 2). They were self-pollinated to produce homozygous recombinants for FHB evaluation and mapping of *Qfhs.ndsu-3AS*. Two homozygous recombinants with the same genotype at the marker loci were identified from the 10 F_3 individuals of 3AS07-42-26 and designated 3AS-Rec II (Table 2). One of these two 3AS-Rec II recombinants was advanced to the F_4 generation for FHB evaluation. Three recombinants heterozygous at different marker loci in the QTL region were identified in the F_3 generation of 3AS07-53-16. Forty F_4 individuals derived from each of these three heterozygous

Table 1 Primer sequences of the STS/SSR markers mapped to 3AS in this study

Markers[a]	Forward primer sequences (5′–3′)	Reverse primer sequences (5′–3′)	Source[b]
Xwgc500	ATATTGGAGTTGCTGTGGAC	TACGCCTACATGTTCTCCTT	TC253371
Xwgc501	CTGGGGCAACTACTTCTACT	AGAAACAAGCCTCAACCAC	TaAffx.516.1.S1_at
Xwgc502	TAGGAAGAGAAACGCGATAG	GTACTGGGTGACGTTGGTAT	TC237031
Xwgc503	CGCCTCCACCATTCTTCTT	GTAGAGCGTGATCACCGTGG	BF293133
Xwgc504	CTGGGTCTACTCCTGTATGG	CGTGGAGGCAAAGATATAAG	TC252511
Xwgc510	GCATACCTCCCTCTCATGTA	TTACAAACATCGTCTGTCCA	TC242060
Xwgc547	CTTTATTTCCGCCACCAC	TCCAGACCCAGCTTAGTAGA	TC254174
Xwgc609	GCAAGTTCTGCTCCCACTTC	ACGACCCAAGAGCATCAAGT	TC267460
Xwgc632	GACCTCAACACCATCGAAGC	GAGCAGGCTGATGTCGAACT	TaAffx.93289.1.S1_at
Xwgc716	CATCTGCTGCAATCCTTGAA	GCTCGGATATCAAACCTCCA	TC255907
Xwgc761	AGGCAGAACCTCAGACACTA	AGGAGAAAGTGGAAAGGAAG	CJ573891
Xwgc774	TGAAGATGGTGAGGATGATG	TGGAGTGCTCTTCTGACAAA	TC254977
Xwgc796	ATGACAGGCCGACAATGAG	TCAACCAATTAAGCAGTTGGAG	TC257223
Xwgc802	AAGAGCGAGGAGAAGAAGAC	ACACACACAACTCGAAGACA	TC238553
Xwgc803	GGCACGTGTCCTTCTCCTAC	CGCTGCCAATACAGCTAACA	TC263447
Xwgc810	CAGGAACTCTGTGAAGAAGG	GAATCGGAGGAGAAAAGAAT	TC259639
Xwgc811	AGAGCTCGCTCAAGGACAAG	GTAGTCGGCCGTCAACATTT	TC248298
Xwgc819	GGTACCTCGAGTACAGCATC	CTCCACCAAGAAAGAAAATG	TC242796
Xwgc826	GGCTGTCGTGGAAGAAGAAG	AAGGCGATGAACACCAAAAC	TC254539
Xwgc858	GTTGGGGGAGAGCATGAAG	CCTGTTGTGATGCGAAAATG	CJ795235
Xwgc883	TGCAAGAAGACGACACAAGG	GGATGAAGCCAATCTTCCAA	DR734177
Xwgc901	CTTTCCCTCCGGCCTACTAC	CAACATTCGGCACGAGACTA	CJ807010
Xwgc929	CGGACGAGAAGAAGCTCAAG	GCTACAATCCAGGGACCAAA	TC268280
Xwgc969	ATCCGTGTTACCCAAATGGA	GCAACAGCTGCAAATCGTAA	TC265006
Xwgc1094	CTGGTGTCCGTTGTTTCCTT	CACATTGGTCCTCCACACAG	TC264939
Xwgc1100	GGGCAACCAAAAAGACAAGA	TTTCAACCCGCTTCTCAAAG	BE412385
Xwgc1143	TTCCAGATCACTCCTCTCC	TGCATGAAACAAAACAACAT	TC273545
Xwgc1144	TATGCAACCATTGATCTTGA	AACACCAGGAAACTTGACAC	TC247145
Xwgc1188	TCTCGCAACTTGTGATGAAA	GGGTACCAGTAGCTGAAGCA	TC326279
Xwgc1204	TCCTTTCTCTCCAGCAGCAT	CTCCGGTTATCTTCCACCAA	TC260079
Xwgc1226	CACTTGGCAGAGCTCAACAG	AGGAGCAGGAGGAGGATCAC	TC258122
Xwgc1229	GCAGGCTGTAAACTCCTTGA	TAACCGGATCAGGATACGAA	TC354664
Xwgc1231	ATGCCAACAGGAAGGTCTTG	CAGCATGCAAATCTCTGGAC	TC288309
Xwgc1238	CGTCAAGACCATTGCTGAGT	CGACACATTCAACATCTCCA	TC278865
Xwgc1240	GTGATTGGGAAGCCAGACAT	ATATTGGAGGCCTTGTGTGC	TC294980
Xwgc1241	GTTCATCACCCGAGCTCATT	TGTGTGGCTTTCTGCACTTC	TC246390
Xwgc1244	GTAGTGCAAGACCCCAAGGT	GACAGCTCCCTCGTCTGAG	CJ670128
Xwgc1248	CTACCACCAGGAGCAGGAAG	AGATTCAAGGAGGAGCGACA	TC247595
Xwgc1254	AGATGTACCCGGAGACGAAG	GACAGCGTAGCAGCATGTTT	TC344003
Xwgc1263	TTCATCAAGAGGCACGACAG	GCTTTGCCATGTTTCACAGA	TC307328
Xwgc1296	TGGTGACTGATGGGATGGTA	GCTGGGGGAAAGGGTAAAT	TC278201
Xwgc1301	AACATCGCCCAGCAGAAC	CGTCGCAGTGTATTTCATTTG	TC252087

[a] *Xwgc547* and *Xwgc761* are SSR markers, while all others are STS markers

[b] Wheat EST accession numbers in the GenBank, TC numbers in the TIGR *T. aestivum* gene index, and contig accession number in the GrainGenes 2.0

recombinants were genotyped using the 11 markers within the QTL region to recover homozygous recombinants in the QTL region. Eleven individuals were identified as homozygous recombinants from each of the two F_4 populations, which were designated 3AS-Rec III and 3AS-Rec IV, respectively (Table 2). Meiotic recombination events were

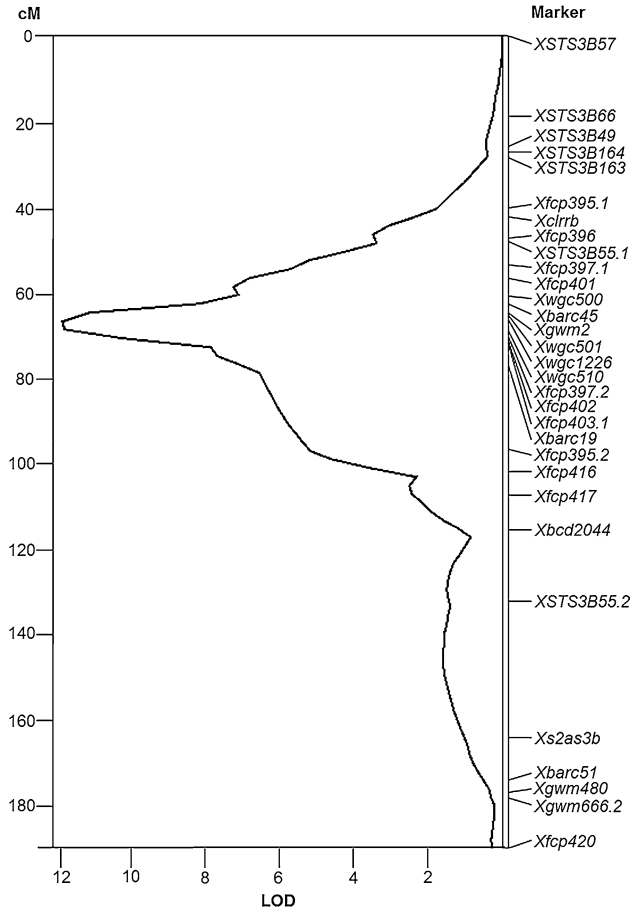

Fig. 2 Composite interval mapping of *Qfhs.ndsu-3AS* against the saturated genetic map in the RICL population. A centiMorgan (cM) scale is indicated to the left and markers to the *right*

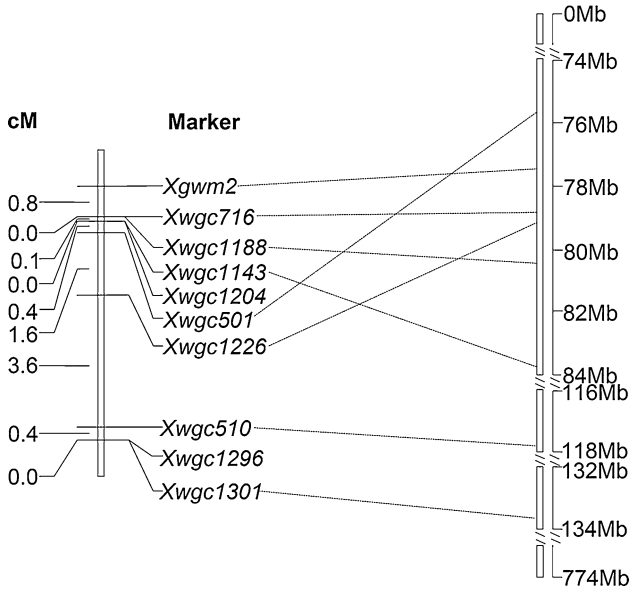

Fig. 3 High-resolution map of the QTL region constructed in the large F_2 population (*left*) and comparative analysis of the QTL region with common wheat 'Chinese Spring' pseudomolecule HG670306.1 on 3BS (*right*)

recovered between *Xwgc716* and *Xwgc1188* and between *Xwgc1143* and *Xwgc1204* in 3AS-Rec III (F_4). A similar recombination event between *Xwgc1143* and *Xwgc1204* was also recovered in 3AS-Rec IV (F_4) (Table 2). *Xwgc716* co-segregated with *Xwgc1188,* and *Xwgc1143* co-segregated with *Xwgc1204* in the F_2 population (Fig. 3). Nine homozygous recombinants were recovered from the third 3AS07-53-16-derived F_4 population and designated 3AS-Rec V (Table 2). In total, five types of homozygous recombinants with shortened *T. dicoccoides* 3AS fragments were generated in the QTL region (Table 2).

The five types of homozygous recombinants at different generations were evaluated for FHB resistance in three greenhouse seasons (Table 3). Nine F_3 individuals of 3AS-Rec I showed FHB severity ranging from 12.0 to 76.9 % in the greenhouse season of Fall 2013. A mean FHB severity of 44.8 % was observed in these nine 3AS-Rec I plants, which was not significantly different from RICL#10, but significantly lower than LDN. Similar FHB severity as 3AS-Rec I was obtained for 3AS-Rec III, IV, and V in Fall 2013. 3AS-Rec II, however, exhibited FHB severity similar to LDN, which was significantly higher than RICL#10 in the greenhouse season of Fall 2013 (Table 3).

The five recombinants selected for FHB evaluation in the first greenhouse season (Fall 2013) were all homozygous at the 11 marker loci within the QTL region. Thus, only one plant of 3AS-Rec I (F_3) and one plant from each of 3AS-Rec III, IV, and V (F_4) were randomly selected for immature embryo culture to accelerate generation. Ten immature embryo-regenerated F_4 plants of 3AS-Rec I had FHB severity similar to LDN in Spring 2014, which was significantly higher than RICL#10. 3AS-Rec II consistently showed a high FHB severity as LDN, while 3AS-Rec III and IV showed FHB severity similar to RICL#10 at the F_5 generation in Spring 2014. F_5 progeny were not obtained from embryo culture for FHB evaluation in 3AS-Rec V (Table 3).

Seed set on the plants with an FHB severity less than 30 % in 3AS-Rec I (F_3), 3AS-Rec III (F_4), and 3AS-Rec V (F_4) were selected for further FHB evaluation based on seed availability in Summer 2014. All three recombinants at the advanced generations (i.e., F_4 for 3AS-Rec I and F_5 for 3AS-Rec III and V) exhibited FHB severity similar to RICL#10. FHB severity of RICL#10, however, was not significantly different from LDN in this greenhouse season. FHB severity of the three recombinants and LDN were lower than other two greenhouse seasons (Table 3).

The genotypes of the three critical RICLs (RICL#10, RICL#15, and RICL#49) and five recombinants

Table 2 Genotypes of the recombinants and their parents at the marker loci in the QTL region

F_2 Rec. sources	Rec. types	*Xbarc45*	*Xgwm 2*	*Xwgc716*	*Xwgc1188*	*Xwgc1143*	*Xwgc1204*	*Xwgc501*	*Xwgc1226*	*Xwgc510*	*Xwgc1296*	*Xwgc1301*
3AS07-39-17	3AS-Rec I	A	A	A	A	A	A	A	B	A	A	A
3AS07-42-26		B	H	A	A	H	H	H	A	A	A	A
	3AS-Rec II	B	B	A	A	B	B	B	A	A	A	A
3AS07-53-16		A	H	H	H	H	H	A	H	A	A	A
	3AS-Rec III	A	A	A	B	B	A	A	B	A	A	A
	3AS-Rec IV	A	A	B	B	B	A	A	B	A	A	A
	3AS-Rec V	A	B	B	B	B	B	A	B	A	A	A
	RICL#10	B	B	B	B	B	B	B	B	B	B	B
	LDN	A	A	A	A	A	A	A	A	A	A	A

A refers to the homozygous genotype of LDN, *B* to the homozygous genotype of *T. dicoccoides*, *H* to the heterozygous genotype

Table 3 FHB evaluation of homozygous recombinants and their parents in three greenhouse seasons

Genotypes	Fall 2013				Spring 2014				Summer 2014			
	No. plants	Gen.	FHB severity (%)	Mean FHB severity (%)	No. plants	Gen.	FHB severity (%)	Mean FHB severity (%)	No. plants	Gen.	FHB severity (%)	Mean FHB severity (%)
3AS-Rec I	9	F_3	12.0–76.9	44.8 ± 22.8b[a]	10	F_4	57.1–85.3	67.0 ± 8.1b	20	F_4	14.6–48.5	27.3 ± 9.5a
3AS-Rec II	9	F_4	43.0–78.6	61.9 ± 13.6c	9	F_4	41.1–87.9	60.1 ± 15.6b	–	–	–	–
3AS-Rec III	9	F_4	14.6–50.3	36.0 ± 10.2ab	8	F_5	21.7–52.0	36.2 ± 10.6a	20	F_5	19.4–54.7	28.8 ± 9.4a
3AS-Rec IV	8	F_4	30.5–72.9	41.2 ± 14.0ab	10	F_5	19.6–59.4	31.4 ± 11.1a	–	–	–	–
3AS-Rec V	8	F_4	9.9–54.2	28.4 ± 14.0a	–	–	–	–	20	F_5	13.6–48.7	26.4 ± 10.5a
RICL#10	12		16.2–73.5	34.5 ± 23.2ab	8		21.1–47.8	30.5 ± 11.9a	6		31.3–40.6	36.0 ± 4.7ab
LDN	12		36.7–79.1	62.3 ± 17.3c	8		60.5–72.4	67.2 ± 5.5b	6		41.0–50.0	47.0 ± 5.2b

[a] Mean ± standard deviation, values followed by different letters are significantly different at $p = 0.05$ level

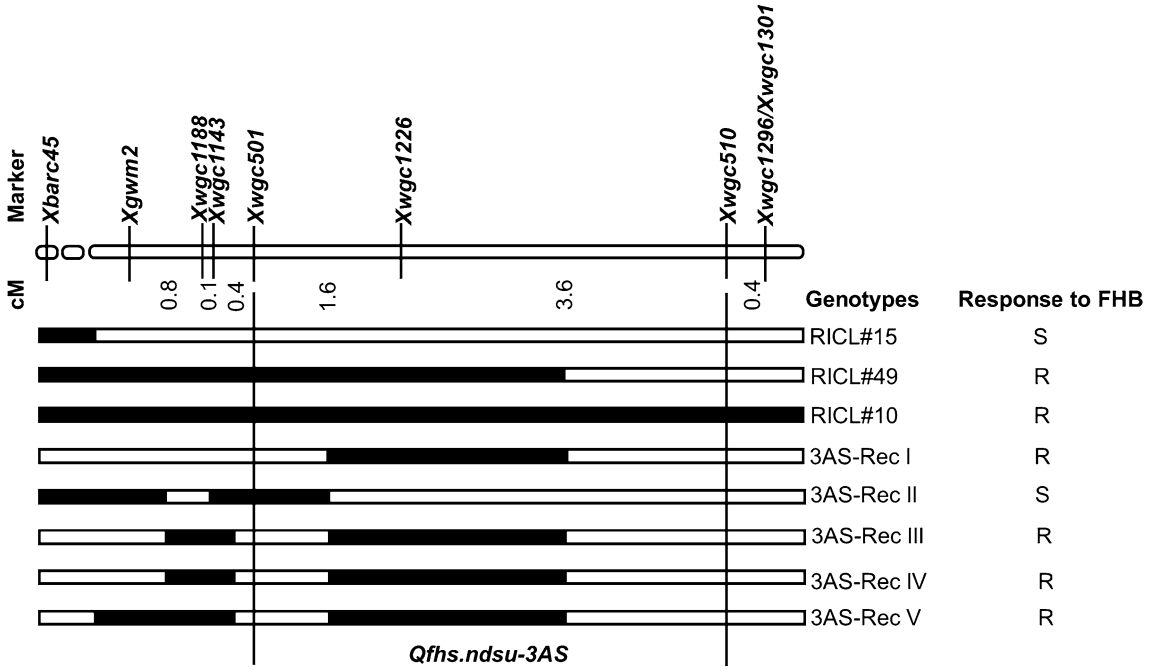

Fig. 4 Graphical genotypes of three RICLs and five homozygous recombinants. Alleles from *T. dicoccoides* and LDN are represented by the *black* and *open boxes*, respectively. The recombination breakpoints were assumed to be in the *middle* of the interval flanked by two markers

homozygous at the marker loci within the QTL region are graphically represented in Fig. 4. Both RICL#10 and RICL#49 were resistant to FHB as their resistance parent LDN(DIC-3A), while RICL#15 was susceptible as the susceptible parent LDN (Chen et al. 2007). RICL#10, LDN, and the five recombinants were evaluated for FHB resistance in 2–3 greenhouse seasons (Table 3). The genotypes with FHB severity significantly lower than LDN were considered resistant and designated "R", whereas those with FHB severity comparable to LDN were considered susceptible and designated "S" (Fig. 4; Table 3; Chen et al. 2007). *Qfhs.ndsu-3AS* was delimited to the chromosomal interval of 5.2 cM flanked by *Xwgc501* and *Xwgc510* based on the genotypes of the RICLs and recombinants at the marker loci within the region and their response to FHB (Fig. 4).

Comparative analysis

The chromosomal interval proximal to *Xgwm2* harbors three groups of co-segregating markers within the QTL region (Fig. 1). The co-segregating markers, including *Xwgc501*, *Xwgc716*, *Xwgc1143*, *Xwgc1188*, and *Xwgc1204*, were 0.6 cM away from *Xgwm2*. These five EST-derived STS markers identified a collinear region of ~154 kb on the short arm of rice chromosome 1 (R1S) and ~100 kb on the short arm of *Brachypodium* chromosome 2 (B2S) (Fig. 1). The other two co-segregating markers, *Xwgc774* and *Xwgc1226*, were 1.2 cM proximal to the five co-segregating markers. These two markers identified a collinear region of ~78 and ~50 kb on rice R1S and *Brachypodium* B2S, respectively. The orientation of the chromosomal interval harboring these two groups of co-segregating markers on 3AS is inverted relative to the corresponding collinear regions on R1S and B2S (Fig. 1). The other three co-segregating markers proximal to *Xwgc774*/*Xwgc1226*, *Xwgc1296, Xwgc1301,* and *Xwgc510*, detected a collinear region of ~339 kb on R1S and ~330 kb on B2S, both of which are located proximally to the rice and *Brachypodium* collinear regions identified by the five co-segregating markers (Fig. 1). The chromosomal segment distal to the QTL region, defined by *Xwgc1229* and *Xgwc500*, showed good collinearity with a genomic region of ~340 kb on R1S and ~300 kb on B2S. A group of ten co-segregating markers distal to *Xwgc1229* detected two collinear regions on R1S and B2S, respectively. Two of the ten markers *Xwgc901* and *Xwgc1263*, identified a collinear region of ~13 kb on R1S and ~10 kb on B2S. This collinear region on R1S was about 2658 kb away from the terminal end of this chromosome arm. The other eight co-segregating markers detected a collinear region of ~141 kb near the terminal end of R1S. However, the collinear region identified by these eight markers on B2S (~90 kb) was located 2600 kb away from the terminal end of the chromosomal arm and ~150 kb proximal to the collinear region detected by *Xwgc901* and *Xwgc1263* on B2S. The collinear regions on R1S and B2S were in inverted orientations (Fig. 1). Eleven

additional new markers mapped to the chromosomal region farther distal to the QTL region on 3AS. Some of them (*Xwgc503/Xwgc811/Xwgc802* and *Xwgc803/Xwgc819*) co-segregated in the RICL mapping population. These two groups of co-segregating markers and three other markers (*Xwgc810*, *Xwgc826*, and *Xwgc796*) proximal to *Xwgc503/Xwgc811/Xwgc802* detected a collinear region of 244 kb on R1S, which was about 910 kb away from the terminal end of the chromosomal arm. The collinear region identified by these eight markers was about 320 kb long on B2S. High collinearity was detected within these genomic regions, in terms of order and chromosomal locations of the marker loci in the respective genomes (Fig. 1). Apart from the inversion of marker loci, deletions of marker loci on the *Brachypodium* chromosome were also observed. For example, the TC sequences of *Xwgc501* and *Xwgc1301* have orthologous sequences on rice chromosome R1, but not on *Brachypodium* chromosome B2 (Fig. 1).

To estimate the physical size of the QTL region, comparative analysis was performed between the refined QTL region and the released 'Chinese Spring' (CS) chromosome 3B pseudomolecules (Choulet et al. 2014). Contigs were identified from the CS chromosome 3AS-specific survey sequence database (http://urgi.versailles.inra.fr/blast/blast.php) using the wheat EST singletons, TCs, or contig sequences corresponding to the newly developed markers in the refined QTL region (Sehgal et al. 2012). Two identical *T. durum* microsatellite sequence clones, Dwm213 and Dwm2, were identified using the 40-bp primer sequence of *Xgwm2*, an SSR marker closely linked to the *Qfhs.ndsu-3AS* QTL peak (Chen et al. 2007). Contigs corresponding to the microsatellite sequence of *Xgwm2* were identified in the 3AS-specific survey sequence database (https://urgi.versailles.inra.fr/blast/blast.php). The identified 3AS contig sequences were used to search against CS chromosome 3B genomic sequences and hit the pseudomolecule HG670306.1 on 3BS. Eight of the ten markers encompassing *Qfhs.ndsu-3AS*, including *Xgwm2*, *Xwc1188*, *Xwgc716*, *Xwgc1143*, *Xwgc501*, *Xwgc1226*, *Xwgc510*, and *Xwgc1301*, were identified to have anchor points in the 3BS pseudomolecule. Those markers, except *Xwgc501* and *Xwgc1226*, maintained the same order in the QTL region on 3AS and its collinear counterpart on 3BS. The collinear counterparts of *Xwgc501* and *Xwgc1226* reside toward the distal region of 3BS relative to those of the markers *Xwgc1143* and *Xwgc1188*, and overlap with the 3BS collinear region identified by *Xgwm2* and *Xwgc716* (Fig. 3). *Xwgc501* and *Xwgc1226* identified a ~3.4 Mb collinear region (75.7–79.1 Mb) in the pseudomolecule. A ~38.8 Mb genomic region in the pseudomolecule was found to be collinear with the chromosome interval flanked by *Xwgc1226* and *Xwgc510* (Fig. 3). Two other markers in the QTL region (*Xwgc1204 and Xwgc1296*) did not detect significant collinearity in the 3BS pseudomolecule.

Discussion

The *T. dicoccoides*-derived FHB resistance QTL *Qfhs.ndsu-3AS* previously mapped to the genomic region (11.5 cM) flanked by two target region amplification polymorphism (TRAP) markers *Xfcp401* and *Xcfp397.2* on 3AS by Chen et al. (2007). Only three markers, including *Xbcd1532*, *Xbarc45*, and *Xgwm2*, mapped within the QTL region (Otto et al. 2002; Chen et al. 2007). In the present study, the *Qfhs.ndsu-3AS* QTL region defined by *Xfcp401* and *Xcfp397.2* was saturated by 22 newly developed wheat EST-derived markers, which stretched the genetic distance of this region to 13.6 cM (Fig. 1). Evidently, the micro-collinearity of the wheat genome with rice and *Brachypodium* provides an effective approach for marker development in saturation and fine mapping of specific wheat genomic regions.

Interval mapping against the saturated genetic map delimited *Qfhs.ndsu-3AS* to a chromosomal segment of 6.2 cM flanked by *Xbarc45* and *Xwgc510*, which is about half the size of the QTL region defined in the previous study (Fig. 1; Chen et al. 2007). Nine of the thirteen markers mapped to the QTL region are newly developed in this study. *Xgwm2*, an SSR marker closely linked to the QTL peak in previous studies (Otto et al. 2002; Chen et al. 2007), still resides within the redefined QTL region. All four intervals defined by the markers within the QTL region explained a predominant phenotypic variation of FHB resistance, suggesting these four intervals encompass *Qfhs.ndsu-3AS* (Fig. 2).

Fine mapping resolved some of the co-segregating markers and stretched map distance between the markers within the QTL region (Figs. 1, 3). The F_2 population employed in this study segregated only at loci within the genomic region harboring the QTL, making it ideal for fine mapping of the QTL. Five meiotic recombinants containing shortened *T. dicoccoides* chromosomal segments within the QTL region defined by *Xbarc45* and *Xwgc510* were recovered in the F_2 and advanced generations of the cross (Fig. 4; Table 2). The recombinant 3AS-Rec I recovered in the F_2 generation exhibited a higher FHB severity than the FHB-resistant parent RICL#10 in the F_3 generation, but the difference was not statistically significant (Fall 2013). In addition, a significantly higher FHB severity than RICL#10 was observed with 10 randomly selected F_4 individuals (Spring 2014). However, the 20 F_4 individuals derived from the F_3 plants selected with FHB severity less than 30 % showed a resistance level similar to RICL#10 (Summer 2014) (Table 3). Apparently, 3AS-Rec I might be still

segregating at *Qfhs.ndsu-3AS* in the F_{2-4} generations even though the markers flanking the QTL were under homozygous conditions. Some of the selected 20 F_4 individuals, if not all, were likely homozygous at *Qfhs.ndsu-3AS* as well as the marker loci within the QTL region.

The recombinants 3AS-Rec III, IV, and V were all derived from the same F_2 individual (3AS07-53-16) that was heterozygous at six of the 11 marker loci in the QTL region (Table 2). These three recombinants were recovered in the F_4 generation. They consistently exhibited a resistance level similar to the FHB-resistant parent RICL#10 across the three seasons (Table 3). Most likely, they were homozygous at *Qfhs.ndsu-3AS* in addition to the marker loci in the region. The recombinant 3AS-Rec II showed a high FHB severity as the susceptible parent LDN in both seasons, suggesting absence of *Qfhs.ndsu-3AS* in 3AS-Rec II (Table 3).

The four FHB-resistant recombinants (3AS-Rec I, III, IV, and V) and two FHB-resistant RICLs (#10 and #49) all contained the *T. dicoccoides* allele at the *Xwgc1226* locus, whereas the susceptible recombinant 3AS-Rec II contained the LDN allele at this marker locus. In addition, the four FHB-resistant recombinants had the LDN alleles at the two marker loci (*Xwgc501* and *Xwgc510*) flanking the *Xwgc1226* locus, whereas 3AS-Rec II had the *T. dicoccoides* allele at the *Xwgc501* locus. Such a genotype at the *Xwgc1226* locus was not observed at other marker loci within the QTL region in the RICLs and recombinants, suggesting *Qfhs.ndsu-3AS* resides within the chromosomal interval defined by *Xwgc501* and *Xwgc510* (Fig. 4).

Comparative analysis suggested that the collinear counterparts of *Xwgc501* and *Xwgc1226* were 3.4 Mb apart on 3BS; and the collinear counterpart of *Xwgc510* was 38.8 Mb away from that of *Xwgc1226* on 3BS (Fig. 3). Thus, the collinearity-based estimate for the physical size of the QTL region defined by *Xwgc501* and *Xwgc510* could be around 42 Mb on 3AS. This estimate, however, could fluctuate because of the complex collinearity of *Xwgc501* and *Xwgc1226* with their counterparts on 3BS in terms of their order relative to other markers in the region (Fig. 3). In addition, this estimate suggests a much higher recombination frequency in the distal segment flanked by *Xwgc1226* and *Xwgc501* (Mb/cM = 3.4/1.6 = 2.13) than the proximal segment flanked by *Xwgc1226* and *Xwgc510* (Mb/cM = 38.8/3.6 = 10.78) within this newly defined QTL region. Meiotic recombination hot spots generally cluster within the gene-rich regions (Faris et al. 2000). Thus, *Qfhs.ndsu-3AS* likely resides closer to the *Xwgc501* locus than to *Xwgc510* based on the distribution of recombination within the QTL region (Fig. 4). Also, this is consistent with the interval mapping results of the QTL, i.e., a higher LOD score with the *Xwgc501-Xwgc1226* interval than the *Xwgc1226-Xwgc510* interval. All these information will be useful in further characterization of this genomic region and cloning of *Qfhs.ndsu-3AS*. The four FHB-resistant recombinants (3AS-Rec I, III, IV, and V) share a shortened *T. dicoccoides* chromosomal interval that contains the resistance QTL *Qfhs.ndsu-3AS*. This reduces potential *T. dicoccoides* 3AS-associated deleterious effects on agronomic traits. They should be breeding-friendly and can be utilized directly in durum wheat breeding for variety development.

Collinearity among the genomes of cereal crops and their models has been extensively investigated (Moore et al. 1995). Wheat homoeologous group 3 chromosomes are collinear to rice chromosome R1 and *Brachypodium* chromosome B2 (Moore et al. 1995; the International Brachypodium Initiative 2010; Sehgal et al. 2012; Luo et al. 2013). In this study, almost all STS/SSR marker sequences mapped on wheat 3AS were found to have orthologous counterparts on both R1 and B2 (Fig. 1). R1 had a higher collinearity with B2 than with wheat group 3 chromosomes in the distal regions of the short arms in terms of the order of the marker loci (Fig. 1). However, the similarity between the genomic sequences of wheat and *Brachypodium* was found to be higher than that between wheat and rice in the collinear regions according to the BLAST search results (data not shown). Thus, *Brachypodium* is more closely related to wheat than rice based on DNA sequence similarities in the genomic regions on the short arms of these three collinear chromosomes. These results are consistent with the observations reported in the previous studies (Catalan and Olmstead 2000; Draper et al. 2001; Foote et al. 2004; the International Brachypodium Initiative 2010; Zhang et al. 2013). However, higher collinearity between wheat and rice than between wheat and *Brachypodium* has been observed in specific genomic regions (Faris et al. 2008). To date, many genes in wheat have been well characterized through comparative analysis with rice and *Brachypodium* genomic sequences (Liu et al. 2003, 2006; Bossolini et al. 2007; Faris et al. 2008; Somyong et al. 2011; Zhang et al. 2013). Comparative analysis of the *Qfhs.ndsu-3AS* QTL and nearby regions on 3AS tremendously enhanced genetic as well as physical mapping of the region in this study. The micro-collinearity revealed in this study will facilitate further understanding of this genomic region.

Conclusions

The genomic region of the wild emmer wheat-derived FHB resistance QTL *Qfhs.ndsu-3AS* was saturated by 42 newly developed wheat EST-based STS and SSR markers. *Qfhs.ndsu-3AS* mapped to a shortened wild emmer chromosomal interval in a higher resolution and was redefined by newly developed markers. The QTL region had a complex

collinearity with rice and *Brachypodium*. The micro-collinearity revealed in this study will enhance further understanding and characterization of the QTL and nearby genomic regions on 3AS. The molecular markers defining *Qfhs.ndsu-3AS* and FHB-resistant recombinants containing the QTL on the reduced wild emmer chromosomal segments will facilitate utilization of this resistance QTL in durum wheat breeding.

Author contribution statement XC and XZ conceived and designed the experiments in this study. XZ performed this study and drafted the manuscript. XC made major revision of the initial draft. SZ provided inoculum for FHB disease evaluation. SC helped in marker genotyping. YQG participated in *Brachypodium* sequence analysis for comparative mapping. SK provided part of the phenotyping data for QTL analysis. EE was co-PI of the grant that financially supported this research project. All authors reviewed, edited, and approved the manuscript for publication.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical standards This study complies with the current laws of USA.

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