ISSN 0026-8933, Molecular Biology, 2008, Vol. 42, No. 4, pp. 504–513. © Pleiades Publishing, Inc., 2008.
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GENOMICS. TRANSCRIPTOMICS. PROTEOMICS

UDC 577.21

Single-Strand Conformational Polymorphism Markers Associated with a Major QTL for Fusarium Head Blight Resistance in Wheat[1]

G. H. Yu[a,b], H. X. Ma[b], G. H. Bai[c], and K. X. Tang[a]

[a] *Plant Biotechnology Research Center, Fudan-SJTU-Nottingham Plant Biotechnology R&D Center, School of Agriculture and Biology, Shanghai Jiao Tong University; Shanghai 200030, China*

[b] *Institute of Agricultural Biotechnology, Jiangsu Academy of Agricultural Sciences, Nanjing 210014, China*

[c] *USDA-ARS, Plant Science and Entomology Research Unit, 4008 Hrockmorton Hall, Manhattan, KS 66506, USA*
e-mail: mahx@jaas.ac.cn; kxtang@sjtu.edu.cn

Received June 26, 2007; in final form, December 03, 2007

Abstract—A major quantitative trait locus (QTL) associated with resistance to *Fusarium* head blight (FHB) was identified on chromosome 3BS between simple sequence repeat (SSR) markers *Xgwm389* and *Xgwm493* in wheat “Ning 7840", a derivative from "Sumai 3". However, the marker density of SSR in the QTL region was much lower than that required for marker-assisted selection (MAS) and map-based cloning. The objective of this study was to exploit new markers to increase marker density in this QTL region by using single-strand conformational polymorphism (SSCP) markers developed from wheat-expressed sequence tags (ESTs) on 3BS bin 8-0.78-1.0. Sixty-nine out of 85 SSCP primer pairs amplified PCR (polymerase chain reaction) products from the genomic DNA of "Chinese Spring”. Thirty-four primer pairs amplified PCR products that could form clear ssDNA (single strand DNA) bands through denaturation treatment. Ten SSCP markers had polymorphisms between Ning 7840 and “Clark”. Five of the ten polymorphic SSCP markers were located on chromosome 3B by nullitetrasomic analysis. Three SSCP markers (*Xsscp6, Xsscp20*, and *Xsscp21*) were mapped into the region between *Xgwm493* and *Xgwm533* and possessed a higher coefficient of determination (R^2) than *Xgwm493* and *Xgwm533*. The SSCP markers, *Xsscp6*, *Xsscp20*, and *Xsscp21*, can be used for map-based cloning of the QTL and for marker-assisted selection in FHB resistance breeding.

DOI: 10.1134/S0026893308040043

Key words: *Triticum aestivum, Fusarium* Head Blight, QTL tagging, SSCP

INTRODUCTION

Fusarium head blight (FHB), caused mainly by *Fusarium graminearum* is a destructive disease of wheat (*Triticum aestivum*) worldwide [1]. From 1993 to 1997, several severe FHB epidemics caused extensive economic losses in North America, with 1993 alone surpassing 1 billion dollars in the United States [2]. In China, one quarter of the wheat growing area (about 6.7 million hectares) is being jeopardized by FHB [3]. Fusarium head blight causes not only considerable yield losses but also decreases grain quality [4]. In addition, wheat grains contaminated with mycotoxins have a major effect on human heath and livestock [4]. The application of fungicide may reduce the severity of the disease, but it inevitably leads to higher cost for wheat production and more environmental contamination. Developing new wheat varieties with a high level of FHB resistance is the most economic and effective measure for the disease control. However, significant interaction between genotype and environment complicates the phenotypic evaluation of FHB resistance and makes screening of FHB resistance laborious, time consuming, and costly [5]. Marker-assisted selection may provide an alternative method for effective selection of the resistance to FHB and speed up the breeding progress.

The Chinese wheat cultivar Sumai 3 and its derivatives have been widely used as the FHB-resistant sources in breeding programs worldwide. Great progress has been made in mapping of QTL for FHB resistance in Sumai 3 and its derivatives. A major QTL for FHB resistance was identified in Ning 7840, a derivative from Sumai 3, with a major effect on reduce FHB severity by using AFLP markers [6]. This QTL was further located on the short arm of chromosome 3B. The QTL was flanked by two SSR markers,

[1] The text was submitted by the authors in English.

Xgwm389 and *Xgwm493* [7]. One of the AFLP markers associated with this QTL was converted into a sequence tag site (STS) marker [8]. However, the STS marker is a dominant marker and amplifies a PCR fragment only in susceptible cultivars. Therefore, it is difficult to distinguish resistant genotypes from failure of PCR amplification when it is used for marker-assisted selection (MAS) in wheat breeding programs. Other STS markers in this QTL region have also been developed from ESTs [9], but only 3BSSTS–256 showed tight linkage with the QTL. Unfortunately 3BSSTS-256 has the same problem as the marker developed by Guo et al. [8].

Therefore, more closely linked markers in this region are urgently needed.

Single-strand conformational polymorphism (SSCP), developed by Orita et al. [10], is a simple, codominant, economical marker system for finding polymorphisms within a DNA sequence [10]. Polymorphisms are due to alterations of mobility induced by nucleotide differences that cause stable changes in conformation of the single strand DNA (ssDNA). SSCP is a PCR-based marker system that can be detected in the same equipment as that for SSR, but much higher DNA polymorphism can be identified by SSCP than by SSR markers. Single nucleotide change, insertion, or deletion (indel) will lead to the alteration of the conformation of the ssDNA. Therefore, it can be a useful system for detection of single feature polymorphism (SFP) between genotypes for developing a high-throughput marker system for marker-assisted selection.

Although wheat genome has not be sequenced, expressed sequence tags (EST) are available for genome mapping in wheat. The ESTs mapped to a specific chromosome region are good resources for identifying markers linked to a QTL on the same region. Over 150 ESTs have been mapped to the wheat bin 3BS 8-0.78-1.0 (http://wheat.pw.usda.gov/NSF), where the major QTL for FHB *Qfhb_3BS* was located [7, 11]. Wheat ESTs on this wheat chromosome bin can be a good source of ESTs for developing SSCP markers. In this study, we used the ESTs from this bin to develop SSCP markers tightly linked to *Qfhb_3BS*.

EXPERIMENTAL

Plant materials. The mapping population consisting of 128 $F_{8–11}$ recombinant inbred lines (RILs) was developed by a single seed descendant from the cross of Ning7840 and Clark [6]. Ning7840 is a wheat cultivar derived from the cross Avrora/Anhui 11//Sumai 3 from Jiangsu Academy of Agricultural Sciences, Nanjing, China. The cultivar possesses high resistance to the spread of FHB within an inoculated spike (type II resistance). Clark is an FHB-susceptible soft red winter wheat cultivar released from Purdue University, West Lafayette, IN, United States. For physical mapping of the SSCP markers, Chinese Spring and its nullitetrasomic lines Nulli (N) 3B Tetra (T) 3A, N3AT3B, and N3DT3A were obtained from the Wheat Genetics Resource Center, Kansas State University, Manhattan, KS, United States. Phenotypic data from Bai et al. used for QTL analysis were the same as that used in [6].

Polymerase chain reaction (PCR). DNA was extracted from young leaves of individual plants by using the cetyl trimethyl ammonium bromide (CTAB) method [12] with minor modifications. The PCR was performed in 20-μl volume containing 10 mM Tris-HCl pH 8.8; 50 mM KCl; 20 mM $MgCl_2$; 200 μM each of dATP, dGTP, dTTP, and dCTP; 1 μl Taq DNA polymerase (Sangon Co.); 20 ng primer pairs (Sangon Co.); and 40 ng template DNA. PCR was carried out in a PTC–100^{TM} thermal cycler (MJ Research, INC.) programmed as follows: 5 min at 94°C, then 40 cycles of 45 s at 94°C, 45 s at 55–68°C depending of primers, and 45 s at 72°C with a final step 10 min at 72°C.

SSCP conditions. SSCP was analyzed by the protocol described by Plomion et al. [13] with modifications. Briefly, 3 μl of PCR products were added to 5 μl of the denaturing loading buffer containing 95% formamide, 10 mM EDTA (pH 8.0), 0.025% of xylene cyanol FF, and 0.025% of bromophenol blue. The samples were heat denatured for 15 min in 98°C and quickly cooled on ice for at least 5 min. The denatured PCR products were separated on 0.4-mm SSCP gels using nondenatured polyacrylamide gel (Acr: Bis was 29:1). Electrophoresis was run in 0.5× TBE buffer at various electrophoretic conditions, such as different gel concentrations, temperatures, and voltage to run a gel. The PCR products were visualized by silver staining [14].

Statistical analysis. SSCP data from this study were combined with SSR data and STS data obtained in the previous studies [7, 8] to construct a linkage map by using Map Manager QTXb20 software, Manly et al. [15]. The software was also used to map the QTL for the resistance to FHB using the same disease data from Bai et al. [6].

RESULTS

Optimization of SSCP Conditions

The SSCP technique is easily affected by several factors [16, 17]. The temperature used for running SSCP electrophoresis is one of the important factors for successful development of SSCP [18]. Three different temperatures were tested for electrophoresis: 4°C, 25°C, and 25°C with the precooled electrophore-

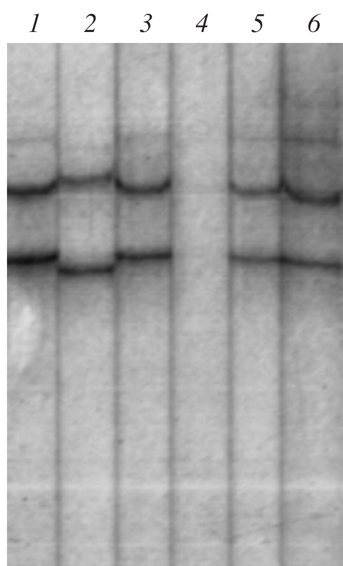

Fig. 1. ssDNA patterns of the *Xsscp6* marker with the DNA of Ning 7840 (lane *1*), Clark (lane *2*), Chinese Spring (lane *3*), N3BT3A (lane *4*), N3AT3B (line *5*), and N3DT3A (lane *6*).

sis buffer at 4°C. The result showed that the best resolution appeared when electrophoresis was run at 25°C with the electrophoresis buffer precooled to 4°C. The reason might be that the electrophoresis buffer precooled to 4°C could keep ssDNA separate at the beginning of the electrophoresis, and then 25°C electrophoretic temperature could form stable conformation for ssDNA. Three different gel concentrations, 8, 12, and 16%, were tested and the result indicated that 12% polyacrylamide gel with Acr : Bis 29 : 1 showed the best result. Based on the result from different combinations of electrophoretic conditions, the best resolution can be achieved by using 12% polyacrylamide gel without glycerol and running at 25°C in precooled 0.5× TBE buffer at 4°C at a constant power of 80 W. This optimized SSCP analysis condition was used for the SSCP markers developing in this study.

Identification of SSCP from Wheat ESTs

About 150 wheat ESTs have been assigned to chromosome deletion bin 3BS 8-0.78-1.0 where the major QTL for the FHB resistance is located (http://wheat.pw.usda.gov/NSF). Based on the EST sequences, 85 primer pairs were designed using the PerlPrimer program (http://perlprimer.sourceforge.net/). A total of 69 primer pairs amplified DNA fragments from the genomic DNA of Chinese Spring. Most of the primer pairs amplified multiple DNA fragments. This was expected because common wheat is a hexaploid consisting of A, B, and D genomes, and the SSCP primer pairs may derive from wheat ESTs that belong to multigene families. However, for SSCP analysis, more DNA fragments will form more ssDNA bands, which make ssDNA bands faint and hard to be distinguished. In this study, those primers that amplified one or two bands were chosen for further marker analysis. Thirty-four primer pairs formed clear ssDNA bands, among them ten primer pairs showed polymorphism between Ning 7840 and Clark (Table 1).

SSCP Associated with Qfhb_3BS

Five of the ten polymorphic SSCP between the parents were assigned to chromosome 3B by the means of nullitetrasomic lines N3BT3A, N3AT3B, and N3DT3A (Fig. 1). These five SSCP markers were used for genotyping the RILs from the population of Ning 7840/Clark. The SSCP data were combined with previously generated SSR data [7, 8] to construct a linkage map. It was shown that four out of the five SSCP markers were mapped in the 3BS FHB-resistance QTL region flanked by SSR markers *Xgwm493* and *Xgwm389* [8]. One marker was located outside this region at 4 cM near *Xgwm389* (Fig. 2).

Three SSCP markers (*Xsscp6*, *Xsscp20*, and *Xsscp21*) have higher coefficient of determination (R^2) and LRS (likelihood ratio statistic) values than the most significant marker, "Xgwm533", previously reported by Zhou et al. [7] (Table 2). In the $F_{6:7}$, the R^2 values for *Xsscp6*, *Xsscp20*, *Xsscp21*, and *Xgwm533* were 48, 44, 47, and 43% respectively. In the $F_{9:10}$, the R^2 values for *Xsscp6*, *Xsscp20*, *Xsscp21, and Xgwm533* were 50, 47, 46, and 45%, respectively. *Xgwm493*, *Xgwm533*, and *Xgwm389* were the most often used markers in marker-assisted selection in marker-assisted breeding for FHB resistance. The new SSCP markers (*Xsscp6*, *Xsscp20*, and *Xsscp21*) obtained in this study can be used for map-based cloning of the QTL and for marker-assisted selection in FHB resistance breeding.

DISCUSSION

SSCP is a PCR gel-based, codominant, high polymorphic marker system. Compared with other DNA markers such as RFLP, AFLP, RAPD, SSR, and STS, SSCP markers reflect the smallest changes (SNPs and indels) in the DNA sequence of the amplified fragments. They could detect more minor differences than other DNA markers. Due to its particular properties, SSCP has been widely used in mutant detection [19, 20], comparative genetics [21], candidate gene analysis [13, 22], estimation of allele frequencies in populations [23], identification of allelic variation of a gene [24], genetic map construction [25, 26], fine mapping [27], etc. To date, there were few SSCP applications in wheat genetic and breeding research. The optimized

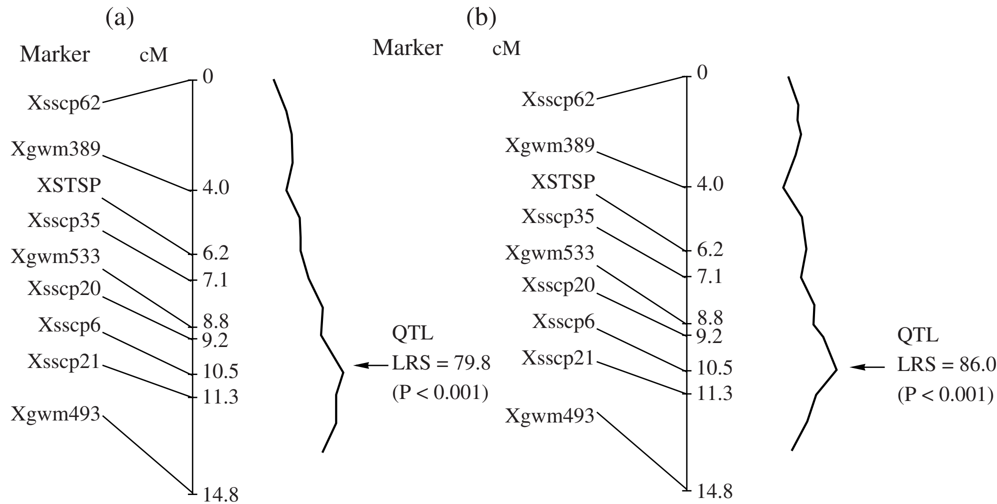

Fig. 2. Interval analysis of FHB resistance associated with chromosome 3BS in the N7840/Clark mapping population using phenotypic data in $F_{6:7}$ (a) and in $F_{9:10}$ (b).

SSCP system reported in this paper could be used in further wheat genetic research.

Over 855600 wheat EST entries are currently available in the EST database of NCBI (National Center for Biotechnology Information). Over 7000 of them have been mapped to specific chromosome deletion bins using Chinese Spring deletion lines (http://wheat.pw.usda.gov/NSF/). In this study, we used the ESTs from 3BS bin 8-0.78-1.02 to develop SSCP markers in order to increase the marker density in the major FHB-resistance QTL region. Four SSCP markers were mapped between SSR *Xgwm493* and *Xgwm389.* With more and more wheat ESTs located on the wheat chromosomes, more SSCP markers will be developed for this major scab resistance QTL using the method reported in this paper. Therefore, this is an efficient strategy to develop DNA markers for the scab resistance QTL region.

The other strategy for exploiting more effective markers of the major scab resistance QTL is to take advantage of the rich rice genomic sequence information and the synteny between rice and wheat. Because wheat chromosome 3BS and rice chromosome 1S are syntenous [28], the sequences of Phage (P1)-derived artificial chromosome (PAC) and bacterial artificial chromosome (BAC) clones covering the subdistal region of rice chromosome 1S could be used as queries for BLASTn searches to identify wheat ESTs that are most likely near *Qfhb_3BS.* Six STS markers were obtained in this QTL region using this strategy in [9]. Compared to STS, SSCP has two advantages. Firstly, SSCP is a codominant marker system. The resistant susceptible parents form different ssDNA bands not like STS markers, which sometimes amplify PCR fragments only in a susceptible parent. So, SSCP is more suitable than STS for map-based cloning of the QTL and for marker-assisted selection in FHB resistance breeding. Secondly, SSCP could detect the polymorphism between two genotypes that have the same base number with one or two different base pairs, while STS usually couldn't. This strategy should also be efficient to develop SSCP markers for the major scab resistance QTL.

Despite extensive efforts to map QTLs for scab resistance in Sumai 3 and its derivatives [6, 7, 29–32], the DNA markers available for marker-assisted selection of scab resistance in wheat are still limited to a few markers, such as *Xgwm533, Xgwm493*, and *Xgwm389.* These markers span a large part of the 3BS chromosome and more closely linked markers to the QTL are needed for fine mapping and cloning of the QTL. Closely linked markers also improve marker-assisted selection efficiency. In addition, these DNA markers may be polymorphic among one set of parents, but not among the others; more markers from the QTL region provide more possible polymorphic markers to be used in marker-assisted selection. In this study, three SSCP markers (*Xsscp6, Xsscp20*, and *Xsscp21*), flanked with SSR markers *Xgwm493* and *Xgwm533*, possessed higher R^2 values than *Xgwm493* and *Xgwm533.* These SSCP markers can be used for high resolution mapping and map-based cloning of the 3BS QTL and also have the potential to be used for high-throughput MAS after conversion of them into single nucleotide polymorphism markers.

Table 1. The GenBank accession no. of wheat ESTs, primer sequences, annealing temperature, and the number of PCR fragments in Chinese Spring, Ning 7840, and Clark of the 85 primer pairs that were designed

no. of primer pairs	EST[a]	Forward primer (5' ⟶ 3')	Reverse primer (5' ⟶ 3')	t_o^b, °C	Number of bands in			SSCP Markers
					Chinese Spring	Ning 7840	Clark	
1	BE446462	TTCTGAAGCTAGTGTTTCTC	ACTAGAACCTAATCTCACCA	55	–	–	–	
2	BE518257	AGGTGCTCAGAGTTGCTTTTGAGGT-GTT	GTACCCTGGGGATGATCTTGAGTA-CATT	68	2	2	2	*Xsscp2*
3	BQ167509	GTCAACAGCTACGTAACCCT	GTGGATGTTCCCTGAGTCTG	60	2	2	2	*Xsscp3*
4	BQ167254	AGTCCACTCCATGATAACAG	CAATCCCATTCTCATTGTCC	55	–	–	–	
5	BQ171072	GACAAGATAGAAAGATCAACGCC	CACGCTGTTGTAAGCATATCTC	65	1	1	1	*Xsscp5*
6	BF293133	AGAAGTATGCCGCCGCCTCCATT	GGTACAGCCCCAGCAGGTAGAACCC	68	1	1	1	*Xsscp6**
7	CD453373	TTCATGGCACCTTCCAAGAC	CTATAGCTTTGGTGTTGTGGT	55	–	–	–	
8	BF484268	CTACTTTCCAGTCAGGACGAGGAG-CAGC	CACCATCACCAAGATCGGAAACA-GAATT	68	3	2	4	
9	BF484398	CTGCCTTCAAATGATTGGAC	TCATAGCATTGTGTCAAGGA	55	–	–	–	
10	BQ167853	GCTCCTAGCAAGTATGTCCC	TTTGCAGAGGTCCTAGATCCA	60	3	2	2	*Xsscp10**
11	CD453551	TCAACGAATCTTAGAACAGTCCAC	CAAACTCGATAGGGATGGCTC	60	1	1	1	*Xsscp11*
12	BE442801	TTCTCCATGCTGATCACGTC	GTTGTTGCAGTCATTCCTACAG	65	3	3	2	
13	BQ162035	CATCACAAGTTAGCATTCAC	TTCATTCAAGAACAGGACAG	55	2	2	2	*Xsscp13*
14	CD454098	ACCTCAGTCTGGTCCATCTC	CATCTGCACTTGCTAACTCTC	65	2	2	2	*Xsscp14*
15	BE442882	CTTCTTACACCGCCTTTCAC	CGGCACTTGTAGTAGTTGGG	60	1	1	2	
16	BF201839	CACTTCTCATCCAAGGATAAGTC	TCACATTGAAGGAGCCAGAG	55	–	–	–	
17	BQ170241	CAGCATCATTTCATGTACCT	CAATATGAATAACTCTGGCTCC	60	2	1	3	
18	BF428807	ATATGGTTCCTCATCAACGA	TACAGTAACTATGGACCCAG	60	2	2	2	
19	CD454892	GCCATCCAAGTTTCCTATAAACTG	GTACGAGAACGATGCAAGGA	60	4	4	4	
20	BE499618	GGATATTGAGCGACTTAATAGG	CGTAGAAACTCGAATGGCAG	60	1	1	1	*Xsscp20**

Table 1. (Contd.)

no. of primer pairs	EST[a]	Forward primer (5' ⟶ 3')	Reverse primer (5' ⟶ 3')	t_o^b, °C	Number of bands in			SSCP Markers
					Chinese Spring	Ning 7840	Clark	
21	BQ169670	ACTCTGAAACAAAGCAGCCT	CTAGATCGTGTGAGAGTCCC	55	2	2	2	*Xsscp21**
22	BQ487452	CAAGTTCAGTGCATATCTTCCA	CTGCCGTCTTTCTTGATGGT	55	–	–	–	
23	BQ171343	AGAGGGTAAAGATGGATGATGAG-TAAGCG	GCACCTCAGAGTCAATAGCACAG-CAGAT	68	1	2	2	
24	CD452790	GGATCAAATCTATCTATGCGGG	GATGTAAGCGAATGGGTGAG	65	1	1	1	*Xsscp24*
25	BF483989	GGAGAATTCCCTATGTGGTG	TCATCATCGTATTGCAAAGG	60	2	2	2	*Xsscp25**
26	BF484355	GAAGCTGATGCAGAAGATCC	AAAGGTGAGTGCTCCAAGAC	60	3	3	2	
27	BQ171064	ACAACACGGGTGAATATAGATCTC	CTTCAGTCTTGGAGCACTCAC	65	6	7	7	
28	BE498112	GCCACCGACTTCTACTCCGAG-GCGTTCG	TGCTCCGCAAGCCGGGCTTTG-TAGTCTC	68	1	2	3	
29	BQ160676	CCCAGGGGAGCATTGCAGAC-GAGAATGGA	GGTTGTTGTTCGGTCGATGAAGC-CACTCGCT	68	3	3	3	
30	BF291730	GAGAACAGTTATTCAAGCACTC	CAAAGGAAGAAAGATGTGGTC	55	–	–	–	
31	BQ159521	CAGACAAACTGTTAGGACTG	GCAACTCAACAATCTATCGT	60	3	3	3	
32	BF291853	CTTGCATGATGAATGGCTGG	GCTCCTTATATTGTATCGCTCGT	65	2	2	3	
33	BF292454	CGGATATGTACAAGAAAGTGGAGG	CTCTTCTGATACGTCGCACTC	60	2	2	2	*Xsscp33**
34	BQ169542	AAGCAGGGACGTTCATTTC-CTACTTTTC	CAGGGGTTGTTGTGGCACGGGGT-TGTAT	68	2	2	5	
35	BQ159579	CCATCCATACATCTAGTTCATCTG	TTTCGGTCTAGGCATGAAGG	55	2	2	2	*Xsscp35**
36	BE495052	ATTGGCGTTGTCATCGGAGGCTGT-TGTGGA	ATGAGGTTCGTGGGTCGCGGCAC-CTCTTGT	68	3	3	3	
37	BQ159588	GACATTCAACAGGAAGAAGATCAG	GAGGTGTAGAGGACATGTGG	55	–	–	–	
38	BE497169	GCAGCCATTCAAATACAGGA	GTCATACCCAGCAAGAACTC	60	3	3	3	
39	BE404656	CAAAGGTCTACCGAGGACAG	TGTGACGATCTATCTTCCCGA	55	2	2	2	*Xsscp39*
40	BG314067	AATGCCATGTTCCTCTATACCC	CTTTCTCCGTCATCCTCATCC	55	-	-	-	
41	BQ162650	AGAAATCTTCGGTGTCAAGG	AATATGGTCATGAGAAGCCTG	55	5	5	5	
42	BE499327	AGATCTCCTGTCTTTCCGTC	CACATAGTCCTCACACGCTC	65	1	1	1	*Xsscp42*
43	BE499327	GGGCCTGTAGATAGGTTCCA	CACATAGTCCTCACACGCTC	55	2	2	2	*Xsscp43**
44	BE488378	CTCAGCTATTTCTTGTCACATCTC	ACATCACGCTTTGAAATTCGG	65	1	1	1	*Xsscp44*

Table 1. (Contd.)

no. of primer pairs	EST[a]	Forward primer (5' ⟶ 3')	Reverse primer (5' ⟶ 3')	t_o^b, °C	Number of bands in			SSCP Markers
					Chinese Spring	Ning 7840	Clark	
45	BQ166912	GCCAGGAATTACAGAGTACACCGGCTAG	ATGGCTCAATGGTGAATGACTGGAATAT	68	3	4	3	
46	BE604713	AGTTTGACTTTGGTCCAGGCAACTATGA	GAGTCGATCAACTAATCTACTAACACCC	68	1	2	2	*Xsscp46*
47	BQ172109	ACAGTACCATTGTATTTCACGG	GAAAGGTACAAGATCGTATGGG	65	1	1	1	*Xsscp47*
48	BQ172035	GCAACACAAGTTCACAAACGA	CTGGTGTCAACTTGGAACTC	65	2	2	1	
49	BE637769	GGCTCTGAATTCCAACTCAC	AGAGACTTCAAACAACCATCTG	55	2	2	2	*Xsscp49*
50	BE637640	TCCCCGAGAAGGCCGTAAAGCCACTCTT	CGATGGTGCCCATGTCCTTATCCGTGTT	68	2	3	3	
51	BQ162368	TTTCAATGCCCCTTTCATCTTGTTCTCA	TAACTTCAGGGCAGCCATTCAAATACAG	68	3	2	3	
52	BQ169850	GCTGGAGGCCGGAAATAACACGAAGGAG	CTGGCTGTCTTCAGGGAGGCAGAGTGGC	68	3	3	3	
53	BE637850	CAAGACTACGTATTGCTTTGGG	CTTGCCAGAAGAAGCATACTC	65	3	3	3	
54	CD453481	CGATCAGTGGATCTTAGAGCAG	GACAAGCAAGCTACATGCAG	60	2	2	2	*Xsscp54*
55	BQ171899	GTTGCTGATGAACAAGGTGG	GGTCAAGTCCATCCAGATCTC	60	3	3	3	
56	BF292411	TGGGTGAGACACTAAGGGAG	ATGCTGGTTATGATGCTCGG	65	1	1	1	*Xsscp56*
57	CD452499	CGCCGTGGCTAAAGCAGTGGCACAACAT	GGCCCGACTTCGTGAATCCCATAGAGTA	68	6	6	7	
58	BF292479	CGTCAAGGTCGATCCATCAG	GAAGATGAGGCATTGCTGGT	65	5	5	4	
59	BE488378	GAGCCGCAAGTCGCCTGACGCCATTCAA	CGATCCAAGGACTAGCAGCAAGCCTAAA	68	3	4	4	
60	BQ169852	GTGTATGCGCTCCAAACAGACAAACAAA	TTCGGCTAGGAACATGACCAACCAACCAG	68	1	2	2	*Xsscp60*
61	BE496983	TGCTGGTCGCCCTGGTGGCGGCAATGAT	TGCTGGTCGCCCTGGTGGCGGCAATGAT	68	2	3	3	
62	BQ169981	AACCTCTAGTTGTACTCGATTCAC	CTCTTGTGTCCCAAGCTACTC	60	2	2	2	*Xsscp62**
63	BF202637	AACACTGCGAATAATGTCAG	GATTCCCACAATCATCATGG	55	–	–	–	
64	BQ170508	GTTAATGACCAGTTTCTACGAC	AGTATTCACAACACAGAGGA	60	6	6	6	
65	BE605229	CTTCCGCTTCCTAACACCAGCTAAATCGTCG	GCCTCGGGCGTCTCCTTCCAGTCAATCC	68	1	2	2	*Xsscp65*

Table 1. (Contd.)

no. of primer pairs	EST[a]	Forward primer (5' ⟶ 3')	Reverse primer (5' ⟶ 3')	t_o^b, °C	Number of bands in			SSCP Markers
					Chinese Spring	Ning 7840	Clark	
66	BQ169082	CTTGGTCGTAACGCACGCTTGAAA-CACT	GCAAGAGCGAGGAGAAGAAGAC-GAGGAG	68	7	8	5	
67	BF292335	CCTAACCCATTTCCGTCCAC	CCCTTTGCAGATTGTTACCT	55	–	–	–	
68	BQ159657	AACAAGCAGGCTAAATAGAGG	GAGGTGGTGTACAACGATACTG	65	2	2	2	*Xsscp68*
69	BG274122	CTGAGATCGACAAGCTCGAC	ACGACGTCATAGTTGTACTTGG	65	3	3	3	
70	BQ159785	TCAGAGCTAAATGTGCAACGA	GAGGTGGATCGACTACAAGG	60	3	2	3	*Xsscp70**
71	BF429301	CAATAGGAGGGAAATGCAACTG	GCTTCTCAATTATTGTGCGA	55	–	–	–	
72	BE443202	TACGGGAACCTAGAGCACAT	ACCTTGTCCCACCCACTC	68	1	1	1	*Xsscp72*
73	CD 452761	CTCTCAATTGCAGGTGTTTGG	CATGAGTACAATCTTGACTGGG	60	1	1	1	*Xsscp73*
74	BF473016	TTAAGGAGTACTTGCCGATCAC	AACAGCTATATCAGCACTCC	55	–	–	–	
75	CD453952	CCAGTGAAAGTAAACATCCC	TTAAGCATCGTTTGTGGGAG	55	–	–	–	
76	BF200008	CAACCCAATGACTTCTGCTG	CACCATACAAGTTTAGCAAGTGAC	60	2	2	2	*Xsscp76*
77	BE443995	GCAATAGGAGGGAAATGCAACT-GATAAA	CCTCAACAATAGGGACTCGTGCCA-GAAC	68	7	7	7	
78	BE422466	GCACATCCCTAATAATTAACCC	CCTCATCAACCAGAAATGCC	55	–	–	–	
79	CD453077	GTAGCACAAACACATAACTCTG	CTTCTTTGTCATGTTGCACC	65	6	6	6	
80	BQ166363	CAGAAAGTACCCTGGGGATGATCT-TGAG	GTGACAGGAGGTGCAGTGGT-TGTCTAAG	68	1	1	1	*Xsscp80*
81	BE423249	TATACAGAGCGCCAAACTC-CCAAAAGCA	GACTCGGATTCAGACGACGAGGA-CAATG	68	3	4	4	
82	BQ167254	CGGCCAGGGCCATCCAGCGTTGAA-GAGC	CGCCAGCCTCCAGAACCACGCCA-CAATC	68	2	6	6	
83	BE489782	GGTGGAGCTGCTGGCCGAGACCAA-CATG	GAGACCTGCCGCCCGATCTGCT-TGAAGG	68	1	1	1	*Xsscp83*
84	BE492937	GGGACATTTGGATACATGGCT	GATGGACACGGCTAATTATCTCC	55	–	–	–	
85	BM138503	CGTTCCCAAGTTTTGTTTTCAATAC-CGC	CAGTTGCCGGGTTGATTATCTGAT-GTCC	68	2	2	2	*Xsscp85*

Note: [a] The GenBank accession no. of wheat ESTs.
[b] Annealing temperature.
* The SSCP marker that had ssDNA polymorphisms between Ning 7840 and Clark.
— The PCR amplification absent or the bands faint and hard to be distinguished.

Table 2. Coefficient of determination (R^2) and LRS values of the SSCP and SSR markers associated with FHB resistance in the *Qfhb*_3BS region in the N7840/Clark RIL population"

Marker	$F_{6:7}$		$F_{9:10}$	
	R^2(%)	LRS	R^2(%)	LRS
Xgwm493	37	57.5	33	49.3
Xsscp6	48	79.8	50	86
Xsscp20	44	70.5	47	78.7
Xsscp21	47	77.5	46	71.5
Xsscp35	41	64.8	42	66.8
Xsscp62	33	49.1	40	62.7
XSTSP	39	61.5	39	60.8
Xgwm533	43	70.2	45	74
Xgwm389	36	55	39	60.5

ACKNOWLEDGMENTS

This study was partly funded by the National Natural Science Foundation (30671302), the 863 program (2006AA100102), and international cooperative project (nos. 2006DFA32850, 2004DFA01100) in China.

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