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P.-G. Guo · G.-H. Bai · G. E. Shaner

AFLP and STS tagging of a major QTL for Fusarium head blight resistance in wheat

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Abstract Large-scale field screening for Fusarium head blight (FHB) resistance in wheat is difficult because environmental factors strongly influences the expression of resistance genes. Marker-assisted selection (MAS) may provide a powerful alternative. Conversion of amplified fragment length polymorphism (AFLP) markers into sequence-tagged site (STS) markers can generate breeder-friendly markers for MAS. In a previous study, one major quantitative trait locus (QTL) on chromosome 3BS was identified by using *Eco*RI-AFLP and a recombinant inbred population derived from the cross Ning 7840/Clark. Further mapping with *Pst*I-AFLPs identified five markers that were significantly associated with the QTL. Three of them individually explained 38% to 50% of the phenotypic variation for FHB resistance. Two of them (pAGT/mCTG57, pACT/mCTG136) were linked to the QTL in coupling, and another (pAG/mCAA244) was linked to the QTL in repulsion. Successful conversion of one AFLP marker (pAG/mCAA244) yielded a co-dominant STS marker that explains about 50% of the phenotypic variation for FHB resistance in the population. The STS was validated in 14 other cultivars and is the first STS marker for a FHB resistance QTL converted from an AFLP marker.

Keywords *Triticum aestivum* · *Fusarium graminearum* · Wheat scab · Fusarium head blight · QTL tagging

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P.-G. Guo · G.-H. Bai (✉)
469 Agricultural Hall, Department of Plant and Soil Science,
Oklahoma State University, Stillwater, OK 74074, USA
e-mail: bai@mail.pss.okstate.edu
Tel.: +1-405-744-9608, Fax: +1-405-744-5269

G.E. Shaner
Department of Botany and Plant Pathology, Purdue University,
West Lafayette, IN 47907, USA

Introduction

Wheat Fusarium head blight (FHB), also called scab, is a re-emerging and destructive disease worldwide (McMullen et al. 1997). FHB epidemics not only caused significant yield losses, but also reduced grain quality due to shriveled seeds and deoxynivalenol (DON) and other toxins contamination (Bai and Shaner 1994). Growing FHB-resistant cultivars will most likely be one of the most effective measures to control the disease. Unfortunately, wheat cultivars in the US are mainly susceptible to FHB, and progress in improving FHB resistance of wheat cultivars has been slow, in part due to the complexity of disease-evaluation procedures and the interaction between genotypes and environmental factors. Recently, molecular-marker technologies have been successfully used to identify a major QTL for FHB resistance in wheat cultivar Sumai 3 and its derivatives (Bai et al. 1999; Waldron et al. 1999; Zhou et al. 2000; Anderson et al. 2001; Buerstmayr et al. 2002). Marker-assisted selection may speed up the breeding process, reduce problems with genotype × environment interactions and facilitate combining different resistance genes into a single plant.

For the major FHB-resistance QTL on the short arm of chromosome 3B (3BS), several AFLP markers have been identified, some of which explained a large portion of the phenotypic variation (Bai et al. 1999). However, none of these has yet been used for marker-assisted selection (MAS) in breeding programs, due to its technical complexity and the high cost of sample preparation and marker detection (Prins et al. 2001). An AFLP marker can be converted into an STS marker. Use of STS markers is technically simple and easily handled by people with a basic knowledge of molecular biology in breeding programs. In addition, STS markers are suitable for high-throughput and automation (Reamon-Büttner and Jung 2000). Conversion of AFLP markers into breeder-friendly STS markers should enhance MAS efficiency for FHB resistance in wheat.

Although conversion of AFLP markers into STS markers is a technical challenge (Shan et al. 1999; Prins

et al. 2001), it has been successful in several crops (Meksem et al. 1995, 2001; Decousset et al. 2000), including wheat (Qu et al. 1998; Shan et al. 1999; Parker and Langridge 2000; Prins et al. 2001). Here we report the conversion of an AFLP marker into an STS marker tightly linked to a FHB resistance QTL in wheat.

Materials and methods

Plant materials and AFLP analysis

One hundred and thirty two recombinant inbred lines (RILs) were derived from the cross between FHB-resistant cultivar Ning 7840 and susceptible cultivar Clark by single-seed descent as reported in Bai et al. (1999). DNA was isolated from the seedlings of each parent and the F_9 RILs with the CTAB procedure (Saghai-Maroof et al. 1984). AFLP analysis was performed as described by Bai et al. (1999). Ninety five *Pst*I and *Mse*I AFLP primer combinations were screened for polymorphisms between the two parents (Table 1). Eighteen informative AFLP primers were used for further characterization of the RILs. AFLP markers were named based on a specific primer combination and the molecular weight of polymorphic bands with that primer combination. For example, pAG/mCAA$_{150}$ refers to the polymorphic band with a molecular size of 150 bp amplified by the combination of a *Pst*I primer with AG as selective nucleotides, and a *Mse*I primer with CAA as selective nucleotides.

Disease evaluation and data analysis

Wheat cultivars Ning 7840 and Clark, as well as their F_7 and F_{10} RILs, were inoculated with *Fusarium graminearum* by single floret inoculation. The inoculum source, inoculation protocol and disease data for the population were the same as that reported by Bai et al. (1999). In brief, each plant was inoculated by injecting 1,000 conidia into the central floret of a spike using a hypodermic syringe. To promote infection, the inoculated plants were placed in a moist chamber for 3 days at 23–25 °C and 100% relative humidity. On the 4th day after inoculation, plants were returned to their original positions on the greenhouse benches. The temperature in the greenhouse averaged 25 °C during the day with a range of 19 to 30 °C, and 19 °C at night with a range of 17 to 21 °C. Disease symptoms were recorded as a percentage of symptomatic spikelets at 3, 9, 15 and 21 days after inoculation. The number of spikelets on each inoculated spike was counted on day 21. Disease severity was calculated as the proportion of symptomatic spikelets per inoculated spike at 21 days after inoculation, and these data were used for QTL analysis. To validate the STS marker, an additional 14 cultivars were also evaluated for disease severities with the same method (Bai et al. 2001).

*Pst*I-AFLP (Vos et al. 1995) data from this study and *Eco*RI-AFLP data generated in a previous study (Bai et al. 1999) were combined to construct a linkage map by using the MAPMAKER program (Macintosh Version 2.0, Lander et al. 1987) with the Kosambi mapping function (Kosambi 1944). For QTL analysis, simple regression and interval mapping analysis was performed by using the QGene program (Nelson 1997). A logarithm of odds (LOD) threshold of 3 was used to claim genetic linkage among molecular markers and the presence of a QTL in a linkage group.

Cloning and sequencing of AFLP bands

The ^{33}P-labelled AFLP bands associated with the FHB-resistance QTL were excised from a dried polyacrylamide gel, and re-hydrated for 3 min in 1 ml of water. The water was decanted and the gel was crushed using a pipette tip. A total of 200 µl of deionized H_2O was added. A microfuge tube containing the DNA was immersed in boiling water for 3 min. The supernatant was transferred to a new tube for further PCR amplification after being centrifuged at 14,000 rpm for 10 min. To verify the size of the target band, the isolated DNA was re-amplified with the original AFLP primers. PCR was conducted in a volume of 24 µl containing 0.2 µM of each primer, 200 µM of each dNTP, 1.7 mM of $MgCl_2$, 1 × PCR buffer, and 0.3 u of *Taq* polymerase under the following conditions: 2 min denaturation at 94 °C followed by 28 cycles of 30 s at 94 °C, 30 s at 55 °C and 1 min at 72 °C, and a final step of 5 min at 72 °C for final PCR extension. PCR products were analyzed on a 6% denatured polyacylamide gel with the original AFLP reactions as a control. If other bands were amplified in addition to the target band, the target band was cut out again, and the DNA extraction process and gel analysis was repeated. If re-amplified PCR generated only one band of the correct size, the PCR product was used for a further cloning process. To increase the product yield, an additional round of PCR was performed with the conditions as described above.

PCR products containing the expected DNA band were ligated into the pT-Adv vector (Clontech Laboratories, Inc. Palo Alto, Calif.), and transformed into DH5α (*Escherichia coli*) competent cells. After transformation, 40 white colonies from each transformation were selected and separately cultured overnight in test tubes containing 5 ml of LB media with 50 µl/ml of Kanamycin. A 22-µl aliquot of each cultural solution was used as template for PCR in a 50 µl volume containing 1 × PCR buffer, 1.5 mM of $MgCl_2$, 240 µM of each dNTP, 0.26 µM of each M13 forward and reverse primers, and 0.2 u of *Taq* polymerase. Thermocycling conditions consisted of an initial step of 95 °C for 1 min followed by 45 cycles of 45 s at 94 °C, 45 s at 53 °C and 1 min at 72 °C, and a final step of 10 min at 72 °C. PCR products were visualized on a 2% agarose gel stained with ethidium bromide. Eleven clones were selected for sequencing based on the size of their PCR inserts in the gel.

The plasmid DNA from the selected clones was isolated using the QIAprep spin Miniprep Kit (Qiagen, Valencia, Calif.) and sequenced with a Li-Cor DNA Analyzer using the Thermo Sequenase DYEnamic Direct Cycle Sequencing Kit with 7-deaza-dGTP* (Amersham Pharmacia Biotech, Piscataway, N.J.). Sequences with at least two identical copies were selected to design primers for further analysis. The primers, amplified by a PCR product cosegregating with the original AFLP marker, was considered as target STS primers. The STS primers were initially utilized to amplify the genomic DNA from the two parents (Ning 7840 and Clark), with PCR components as described above, and PCR conditions as following: 2 min at 94 °C followed by 38 cycles of 30 s at 94 °C for denaturation, 30 s at 45–67.5 °C for annealing, varied with the Tm of different primer sets, and 1 min at 72 °C for extension and by a final extension step at 72 °C for 5 min. For AFLP marker pAGT/mCTG57, the PCR-walking as described by Negi et al. (2000) was used to isolate genomic sequences flanking the marker. Restriction enzymes *Eco*RI, *Pst*I and *Mse*I were used for digestion of genomic DNA from Ning 7840 and Clark for PCR-walking.

Table 1 AFLP primers used for pre- and selective-amplification. 'p' and 'm' represent the pre-amplification primers of *Pst*I and *Mse*I, respectively

Pre-amplification primers	
*Pst*I	GACTGCGTACATGCAG
*Mse*I	GATGAGTCCTGAGTAA
Selective amplification primers	
*Pst*I	pACT, pAG, pACTG, pAGT, pGCT
*Mse*I	mACGC, mAGC, mCAA, mCAC, mCACG, mCAG, mCAGT, mCAT, mCGAC, mCTA, mCTC, mCTG, mCTGA, mCTT, mGAC, mGCG, mTGC, mGCAG, mGTG

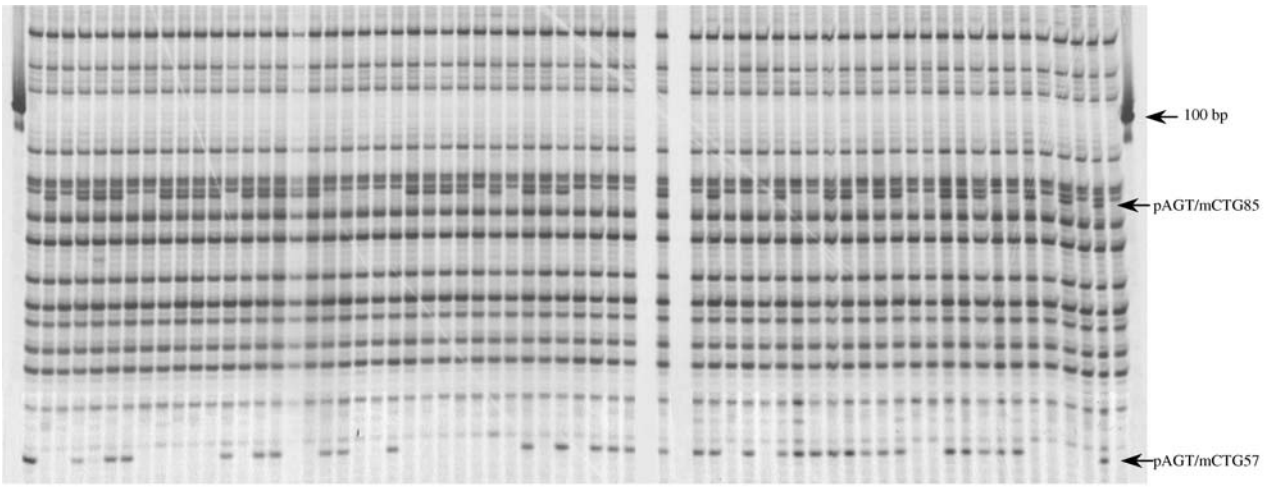

Fig. 1 An AFLP gel showing polymorphic bands segregating among Clark (*second lane* from right), Ning 7840 (*third lane* from right) and their 64 F9 RILs (*lanes 4 to 67* from right). DNA was amplified with pAGT/mCTG primers. *Lanes 1 and 68* are molecular size markers (100-bp DNA ladder from Life Technologies, Inc, USA)

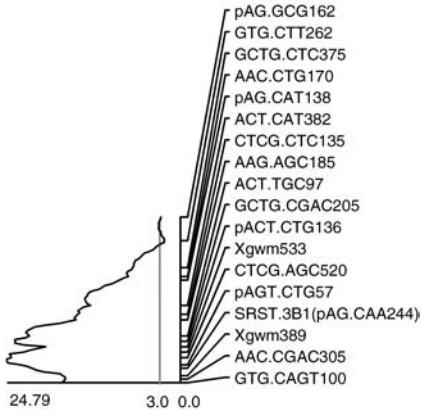

Fig. 2 An AFLP linkage group harboring the major QTL for FHB resistance on chromosome 3BS, showing AFLP markers (right) and LOD peak (left) distribution of the major QTL for FHB resistance over the 3BS region in F7 RILs by using the QGene program (Nelson 1997)

Verification of the STS marker

If a primer set amplified a polymorphic band between two parents, it was further used for segregation analysis in the RILs. To verify that the STS primers amplified the polymorphic bands associated with the FHB resistance QTL, the STS segregation pattern in the mapping population was compared with the data from the original AFLP. To assess the potential for large-scale application of the STS marker in MAS, nine Ning 7840-related cultivars and seven cultivars or breeding lines without Chinese pedigrees were analyzed with the STS marker.

Results

Construction of a high-density map with *Pst*I-AFLPs

Among 95 *Pst*I/*Mse*I AFLP primer combinations screened, about 80 primer pairs amplified a scorable banding pattern. Eighteen of them were further assayed in the RILs. A total of 2,018 bands were amplified, of which 274 polymorphic bands were scored unambiguously (Fig. 1). On average, 15 polymorphic bands per primer pair were scorable, indicating that the multiplex *Pst*I-AFLP can generate a high level of polymorphism in the cross Ning 7840/Clark.

The *Pst*I-AFLP markers were incorporated into the *Eco*RI-AFLP map developed previously (Bai et al., unpublished data). About 320 *Eco*RI-AFLP markers at an interval of about 2 cM were selected for new map construction. A total of 240 *Pst*I-AFLP markers were integrated into the new linkage map (data not shown). *Pst*I-AFLP markers showed random distribution in the new map, and not only filled some large gaps, but also increased marker density in some chromosome regions where *Eco*RI-AFLP markers were already dense.

The LOD peak region of the major QTL for FHB resistance on chromosome 3BS is depicted in the new linkage map (Fig. 2). In this map, five *Pst*I-AFLP markers (pAG/mCAA244, pAGT/mCTG57, pACT/mCTG136, pAG/mCAT138 and pAG/mGCG162) demonstrated significant association with the major QTL for FHB resistance. Three of them (pAG/mCAA244, pAGT/mCTG57 and pACT/mCTG136) explained a large proportion of phenotypic variation with R^2 values of 0.5083, 0.4591 and 0.4961 in the F7 generation, and 0.426, 0.3827 and 0.4278 in the F10 generation, respectively. Among them pAGT/mCTG57 and pACT/mCTG136 were linked to the QTL in coupling and the marker pAG/mCAA244 was linked to the QTL in repulsion.

Development of the STS marker

Since two *Pst*I-AFLP markers (pAG/mCAA244 and pAGT/mCTG57) explained a large portion of phenotypic variation for FHB resistance in repeated FHB tests

and can be easily separated from other AFLP fragments, they were selected for the development of STS markers. The sequencing result indicated that 11 colonies of the AFLP band pAGT/mCTG57 contained an identical 35-bp sequence after removal of the adapter sequences from both ends. However, we were not able to convert the AFLP band into an STS marker due to the lack of polymorphism between the two parents even if the PCR-walking technique (Negi et al. 2000) was used. The sequencing result from the pAG/mCAA244 band showed that all 11 clones had a 244-bp insert including a *Pst*I primer sequence at one end and an *Mse*I primer sequence at the other. However, the 11 clones could be distributed into five distinct sequence groups that consisted of 5 (Fig. 3), 3, 1, 1 and 1 clones, respectively. We selected the first two groups of DNA sequences to design STS primers. Two to three sets of primers with various numbers of nucleotides were designed for each sequence and used to amplify genomic DNA from cultivars Ning 7840 and Clark. Only one set of primers from the first sequence group amplified a single unambiguous polymorphic band between the two parents. This primer pair generated a 212-bp band in Clark, and amplified an additional band of about 190 bp in Ning 7840 (Fig. 4). This primer pair was further used to analyze the RILs. The amplified banding pattern fitted the expected co-dominant segregation ($x^2 = 3.72$, $p > 0.05$) (Fig. 4). The STS marker, designated as SRST.3B1, is the true representative of the original AFLP marker because it showed the same segregation pattern as the original AFLP marker in the RILs. Regression analysis indicated that SRST.3B1 explained 50.02% and 40.18% of the phenotypic variation as reflected by the proportion of infected spikelets in an inoculated spike in the F_7 and F_{10} generations, respectively. A small difference in R^2 values between the STS and the original AFLP is due to missing AFLP data from several RILs. This is the first STS marker derived from an AFLP for the major FHB-resistance QTL on chromosome 3BS of wheat.

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  1  TTAACAAAAGATCCAACGTGTATATAGTACCAATAAAGAGAGATTCAACG
 51  TATCAAACGAGGATGAAATCAAAATCAAGTTTGATGGCAAAAATGAACAT
101  GTGTCAAGGAGTGCCCGGAACCAAAACACCTTTGCCTTGCTGAGTCTTCT
151  GCTAGCTTGTCCATGGCTCTCTCTTTTCTTGTGAGGGTTGTTCGTGGAAC
201  TTTTCATCTACCCCCTCTGCAG
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Fig. 3 Nucleotide sequence of the AFLP marker pAG/mCAA244 from the susceptible cultivar Clark excluding adapter sequences from both ends. *Underlined nucleotides* are the sequences used for designing SRST.3B1 primers

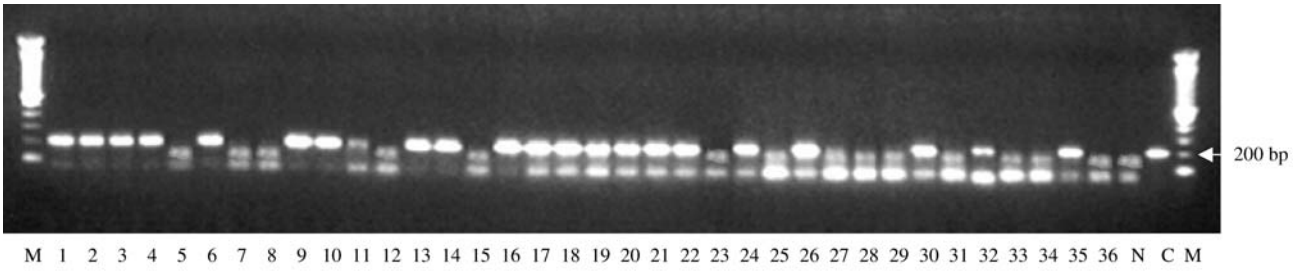

Fig. 4 The PCR products amplified by the STS primer combination showing segregation pattern among RILs. *Lanes 1 and 40* are the molecular size markers (100-bp DNA ladder from Life Technologies, Inc, USA). *Lanes 2 to 39* from left to right are the 36 F_9 RILs from the cross of Ning 7840/Clark, Ning 7840 (*N*) and Clark (*C*). In the gel, total three sizes of bands were amplified in different genotypes: the largest band (212 bp) is the specific STS band for the Clark genotype and second largest band (about 190 bp) is the specific STS band for the Ning 7840 genotype, and the smallest band is a non-target band that was amplified from both parental genotypes. The PCR products were electrophoresed on a 2% agarose gel

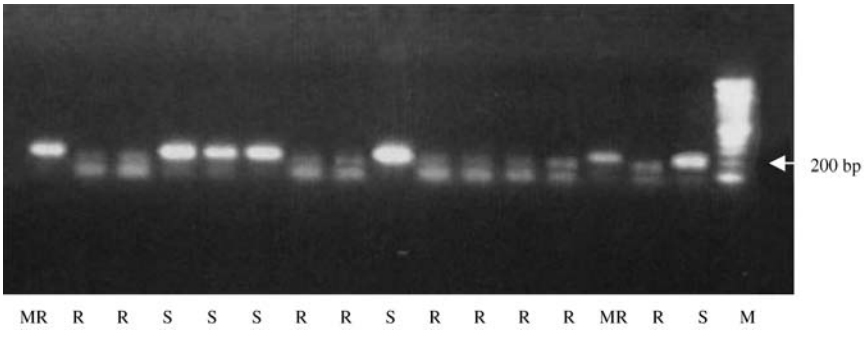

Fig. 5 STS banding patterns amplified in 16 wheat cultivars. *Lanes from left to right* are: cultivars Expert, Fu5114, Ning8331, MO-94-193, IL94-6280, Clark, Sumai49, Dwarf Sumai3, Cardinal, IL9634-24851, Fu5125, Ning8026, Ning7840, IL94-1549, Sumai3, Pontiac and the molecular-size marker (100-bp DNA ladder from Life Technologies, Inc, USA). The PCR products were analyzed on a 2% agarose gel. "R", "MR" and "S" represent FHB resistance, moderate resistant and susceptible lines, respectively

Table 2 Pedigree and proportion of symptomatic spikelets (PSS) per inoculated spike for the 16 wheat cultivars analyzed with the STS marker

Cultivar	Pedigree	PSS
Dsumai3*[a]	Sumai3/Tom Thumb//Tom Thumb	0.08
Fu5114*	LongXi18/Ning 8017	0.06
Fu5125*	Fufan904/Ning 8017	0.05
IL9634-24851*	IL90-6364//IL90-9646/Ning 7840	0.16
Ning 7840	Aurora/Anhui11//Sumai3	0.08
Ning 8026*	Aurora/Sumai3//Yangmai2	0.28
Ning 8331*	Yangmai4/Ning 7840	0.12
Sumai3*	Funo/Taiwanxiaomai	0.08
Sumai49*	N7922/Ning 7840	0.08
Cardinal	Logan *2/3/Va63-5-12/Logan//Blueboy	0.76
Clark	Beau//65256A1-8-1/67137B5-16/Sullivan/Beau//5517B8-5-3-3/Logan	0.94
Expert	Extrem/Mexico4040//Neuhof1/3/Extrem/HP35719	0.37
IL94-1549	Auburn/Ark38-1/Arthur/Blueboy	0.30
IL94-6280	IL87-3721/Cardinal//P808801-4-2-4-107	0.94
MO-94-193	MO 11728/Becker	1.00
Pontiac	Magnum/Auburn	0.71

[a]* Wheat cultivars or lines have the same STS banding pattern as that in Ning 7840

Validation of the STS marker in different genetic backgrounds

To further evaluate the possibility of using the SRST.3B1 marker to predict the major QTL on chromosome 3BS in different genetic backgrounds, we analyzed 14 additional cultivars with various pedigrees. The banding pattern of the SRST.3B1 marker in these cultivars was consistent with their pedigree information (Fig. 5 and Table 2). All resistant cultivars with Ning 7840, Sumai 3 or their sister lines in their pedigrees, amplified the same band as in Ning 7840 (about 190 bp), while susceptible cultivars or cultivars without Ning 7840 or Sumai 3 in their pedigrees amplified a band the same as that in Clark (212 bp). Therefore, this STS marker showed a high level of polymorphism among cultivars commonly used in breeding programs and has great potential to be widely used for marker-assisted selection of the major FHB-resistance QTL on wheat chromosome 3BS.

Discussion

Although wheat cultivars with various degrees of FHB resistance have been identified from several countries, Chinese resistant cultivars such as Ning 7840 and Sumai 3 seem to have both Type II and Type III resistance (Bai and Shaner 1994; Bai et al. 2001). FHB-resistance genes in Ning 7840, and its relatives mainly derived from Sumai 3, have been widely used as a major FHB-resistant source in breeding programs worldwide. Developing molecular markers associated with the FHB-resistance QTL in these cultivars may accelerate breeding progress for FHB-resistance improvement in wheat. Field screening of breeding materials is difficult because of the interaction of flowering-date and weather conducive for infection and symptom expression. Once markers tightly linked to major FHB-resistance genes are developed, they can be used for selection against almost all strains of *F. graminearum* and other *Fusarium* species worldwide since the resistance genes from Sumai 3 have demonstrated a wide spectrum of resistance to various strains of *F. graminearum* and other *Fusarium* species (Bai and Shaner 1994, 1996).

DNA marker technologies such as RFLPs and SSRs have been widely used in genotyping and molecular mapping (Chao et al. 1989; Roder et al. 1998). However, low polymorphism, low throughput, and high cost of RFLP markers make it impossible to use them for MAS in wheat (Chao et al. 1989). In contrast to RFLPs, SSRs are simple and detect a high level of polymorphism in wheat (Roder et al. 1998). Once identified, some of SSRs are well adapted to large-scale, locus-specific application. Unfortunately, the number of SSR primers is still limited in wheat (Roder et al. 1998), and developing SSR primers require fairly sophisticated training and resources. AFLP markers are multiplex and highly polymorphic in wheat (Bai et al. 1999). It is a rich marker source for molecular mapping and QTL tagging of important wheat traits. Using *Eco*RI-AFLP, one major QTL for FHB resistance was identified in a previous study (Bai et al. 1999). Because *Pst*I-AFLPs may access some regions of the genome that are not accessible to *Eco*RI-AFLP, they were further used to saturate the major QTL region in this study. On average, about 15 scorable polymorphic bands segregating in the mapping population indicated a high level of polymorphism of *Pst*I-AFLP in wheat. Among the mapped 240 *Pst*I-AFLP markers, five were located on 3BS, and three were tightly linked to the major QTL (Fig. 2). Because AFLP is a relatively quick assay and provides many primer and enzyme combinations, it is an efficient system for high-resolution mapping of the FHB resistance QTL in wheat.

Although AFLP markers can be readily used for screening breeding materials, they are still poorly adapted to large-scale and locus-specific applications (Bradeen and Simon 1998). STS is simple, rapid and inexpensive, and can be directly used for large-scale screening of breeding materials in MAS programs. Successful conversion of AFLPs into STSs has been reported in several plant species (Lu et al. 1999; Shan et al. 1999; Decousset et al. 2000; Negi et al. 2000; Prins et al.

2001). In wheat, four examples have been reported, but only one of them isolated an STS through segregation analysis (Parker and Langridge 2000). In contrast, all others identified STS markers by comparing euploid wheat profiles with those of deletion mutants or null-itetrasomic lines (Qu et al. 1998; Shan et al. 1999; Prins et al. 2001). In this study, we successfully converted an AFLP into an STS marker closely linked to the major QTL for FHB resistance through segregation analysis. This STS explained about 50% of the phenotypic variation for FHB resistance. This is the first STS marker derived from an AFLP tightly linked to the 3BS major FHB-resistance QTL. Therefore, this marker will be a useful tool for MAS of the major QTL.

In general, conversion of AFLPs into STS markers poses great technical challenges due to the nature of AFLPs (Bradeen and Simon 1998; Prins et al. 2001). First, the AFLP fragment is too short for designing an appropriate PCR primer to amplify a polymorphic band (Bradeen and Simon 1998); and second, different AFLP fragments of the same size may migrate together in the gel, and a target polymorphic band may contain contaminating fragments from adjacent bands (Prins et al. 2001). Several procedures have been proposed to solve those problems (Bradeen and Simon 1998; Negi et al. 2000; Meksem et al. 2001). In this study, we successfully converted a 222-bp AFLP fragment into an STS marker. We saw few contaminating fragments from adjacent bands in the first gel run, but the contaminating bands were eliminated after the second round of DNA isolation. All the 11 sequenced clones contained the same molecular size, indicating that contaminating fragments from adjacent bands can be eliminated through carefully cutting the expected bands from gels twice. This method cannot eliminate the fragments with different sequences but the same molecular size were seen in this study and some other studies (Meksem et al. 2001). To select the right fragment for STS, Prins et al. (2001) recommended sequencing more clones and checking specificity by PCR for all fragments with different sequences. In this study, among 11 clones sequenced, we identified five groups of distinct sequences. We designed several primers based on the two largest groups of sequences, but only one of these primers amplified a polymorphic band between the two parents, suggesting that the sequence representing more clones from an AFLP band may most likely be the original polymorphic band. This is different from the result of Prins et al. (2001) in which an STS was identified from the second smallest sequence group.

The Length of AFLP bands for successful conversion of an AFLP marker into an STS is still equivocal. Meksem et al. (1995) indicated that an STS derived from the 80 to 120-bp AFLP bands was too small to detect polymorphism. However, the smallest fragment used for successful conversion of a co-dominant AFLP into an STS marker was 46 bp (Parker and Langridge 2000). In this study, the attempt to convert a 35-bp AFLP fragment failed to generate polymorphic bands between the parents, even by using the PCR-walking technique (Negi et al. 2000).

Success rates in converting AFLPs into STSs are reasonably high (Bradeen and Simon 1998; Parker and Langridge 2000; Meksem et al. 2001; Prins et al. 2001) with one exception (Shan et al. 1999). In this study, two AFLP bands were investigated and one was successfully converted into an STS. Because AFLPs are reliable and multiplex with unlimited primers, they offer the best available means for uncovering linked DNA sequences in wheat. If AFLPs can be converted into more reliable, cost-effective, high throughput and locus-specific STS markers, they can be directly used to screen breeding materials on a large scale to accelerate the breeding progress. STS markers are also a powerful tool for the creation of isogenic lines for fine structure mapping of QTLs, the introgression of genes into adapted cultivars, or even screening a BAC library to verify or fill gaps in a physical map (Meksem et al. 2001). Therefore, it is worthwhile, in general, to convert AFLP markers into STS markers for marker-assisted breeding in wheat. However, techniques for isolating the true sequence corresponding to the original AFLP marker still need to be improved in order to enhance its convertibility.

The STS developed in this study not only showed a high R^2 value for the major FHB resistance QTL, but also showed polymorphism between several resistant cultivars related to Ning 7840 and unrelated susceptible cultivars in this study. This result suggests that the STS can be used for MAS in diverse genetic backgrounds, and therefore it has great potential to be used for selection of the 3BS QTL in breeding programs worldwide. In addition, consistent and accurate amplification of specific bands using crude DNA has also been demonstrated (data not shown). This STS will offer a simple, rapid, accurate PCR-based assay of the 3BS QTL in the early seedling stage, which can significantly reduce breeding cost, shorten breeding cycles and improve selection efficiency.

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