Theor Appl Genet (2003) 106:1041–1047
DOI 10.1007/s00122-002-1133-8

X. Shen · M. Zhou · W. Lu · H. Ohm

Detection of Fusarium head blight resistance QTL in a wheat population using bulked segregant analysis

Received: 5 July 2002 / Accepted: 12 August 2002 / Published online: 29 October 2002

Abstract A population of 218 recombinant inbred lines (RILs) was developed from the cross of two wheat (*Triticum aestivum L.*) cultivars, 'Ning 894037' and 'Alondra'. Ning 894037 has resistance to Fusarium head blight (FHB) and Alondra is moderately susceptible. Response of the RILs and their parental lines to FHB infection was evaluated with point inoculation in four experiments both in greenhouse and in field conditions. Distribution of disease severity in the population is continuous, indicating quantitative inheritance of resistance to FHB. Bulked segregant analysis and QTL mapping based on simple sequence repeat (SSR) markers revealed three chromosome regions that are responsible for FHB resistance. A chromosome region on 3BS accounted for 42.5% of the phenotypic variation for FHB resistance. Additional QTLs were located on chromosomes 2D and 6B. These three QTLs jointly accounted for 51.6% of the phenotypic variation. SSR markers linked to the QTLs influencing resistance to FHB have potential for use in breeding programs.

Keywords Wheat · Fusarium head blight (FHB) · Quantitative trait loci (QTL) · Simple sequence repeat (SSR)

Introduction

Arthur (1891) reported that a wheat field which was expected to yield 35–40 bushels/acre yielded only 8 bushels/acre in 1890, a season in which there was a severe epidemic of Fusarium head blight (FHB). The disease has frequently caused low to severe wheat crop losses in the United States (McMullen et al. 1997), and with increased frequency and severity coinciding with the recent widespread adoption of reduced soil tillage for purposes of soil conservation and reduced input costs of crop production. It is a floral-infecting disease caused by the fungus *Fusarium* species, with *Fusarium graminearum* Schwabe, telomorph *Gibberella zeae* (*Schw.*) *Petch*, as the predominant causal organism in the U.S. Infected wheat florets and spikelets are often destroyed. The fungus readily colonizes florets, spreading through the rachis to adjacent spikelets. The fungus produces mycotoxins, including deoxynivalenol (DON), causing Fusarium infected grains to be toxic to animals and humans (Snijders 1990a).

Development of FHB-resistant cultivars is an effective way to reduce damage from the disease. However, several factors combine to make selection for resistance difficult. First, the expression of FHB resistance appears complex. At least five types of resistance have been described (Schroeder and Christensen 1963; Miller et al. 1985; Mesterhazy 1995). Second, disease severity, the percentage of diseased spikelets in a spike, shows continuous distributions in segregating populations, as a result of the action of several loci and large environmental effects (Snijders 1990b; Singh et al. 1995; Van Ginkel et al. 1996). Single plant heritability is low (Snijders 1990b), which makes selection in early generations difficult. Third, limited information is available about the allelism of different resistance genes. Some Chinese cultivars possess FHB resistance, but many of them were derived from Sumai 3 such as the Ning series. Further, many of them, including Sumai 3, were thought to inherit resistance from Italian lines (Lu et al. 2001). With the development of molecular markers and recombinant inbred lines, complex traits can be characterized more accurately and the inheritance can be dissected into different genetic components. RFLP mapping has been used to show that there are at least three genes involved in the resistance of 'Sumai 3', a widely used Chinese resistance source (Waldron et al. 1999). A QTL with a

Communicated by J. Dvorak

X. Shen · H. Ohm (✉)
Department of Agronomy, 1150 Lilly Hall, Purdue University, West Lafayette, IN 47907, USA
e-mail: hohm@purdue.edu
Tel.: +1-765-4948072, Fax: +1-765-4962926

M. Zhou · W. Lu
Institute of Genetics and Physiology,
Jiangsu Academy of Agricultural Science, 50 Zhongling Street, Nanjing, Jiangsu, P.R. China

substantial retarding effect on disease spread within a spike (type II resistance) has been identified on the short arm of chromosome 3B in Sumai 3 and its derivatives (Waldron et al. 1999; Zhou et al. 2000; Anderson et al. 2001; Buerstmayr et al. 2002). From the perspective of wheat breeding, the discovery of novel host plant resistance genes is desirable since it will enable gene pyramiding. In this research, we conducted QTL analysis for FHB resistance in a spring wheat population derived from the cross of 'Ning 894037'/'Alondra'. Ning 894037 is a highly resistant line developed from tissue culture (Lu et al. 2000) with the goal of developing somaclonal variation for FHB resistance. Neither Ning 894037 nor Alondra have Sumai 3 in their parentage, but both Ning 894037 and Sumai 3 have the Italian cultivar 'Funo' as a parent. Thus, it was postulated that the resistance of Ning 894037 is either from Funo, or resulted from somaclonal variation due to tissue culture, or both. The objectives of this research were to determine the inheritance of the resistance of Ning 894037, identify the chromosomal location of the resistance, and compare the resistance QTLs with those of other resistance sources that have been reported.

Materials and methods

Mapping population

A population of 218 recombinant inbred lines (RILs) was developed by single-seed descent from a cross of Ning 894037 and Alondra. Ning 894037 was developed at the Institute of Genetics and Physiology, Jiangsu Academy of Agricultural Science, China. It was derived from a tissue-culture protocol using 'Yangmai 3' as the source of cells (Lu et al. 2000). Regenerated plants were screened for FHB resistance in the field for several years. One of the selections, Ning 894037, has stable type II resistance over years and locations (Lu et al. 2000). Alondra was developed at the International Maize and Wheat Improvement Center (CIMMYT), with the parentage D6301/Nainara 60//Weique/Rojal de Murcia/3/Ciano 67 *2/Chris. Alondra has a moderately susceptible response to FHB infection. Ning 894037 and Alondra were selected as the parents for the RI population because they showed high molecular polymorphism based on RFLP analysis, compared with other pairs of cultivars that differed in FHB resistance (Shen et al. 1998).

Since Yangmai 3 is a selection from the Italian cultivar Funo, one of the parents of Sumai 3, it is of interest to determine whether or not the FHB resistance QTL on 3BS in both Ning 894037 and Sumai 3 originated from Funo. Therefore, three accessions of Funo (CItr 14349, PI 213833 and PI 303665) from the USDA, ARS Small Grains and Potato Germplasm Research Unit, Aberdeen, Idaho, were analyzed with the markers flanking the 3BS QTL. Sumai 3 and its other parent, Taiwan Wheat, were also included in the study.

FHB evaluation

The two parent lines, Ning 894037 and Alondra, and the 218 RILs in the $F_{7:8}$ through the $F_{10:11}$ generations were tested for FHB disease spread, following inoculation of a single floret at flowering in four experiments. One field experiment was conducted at the Agronomy Research Center, Purdue University at West Lafayette, Indiana, in May–June 2000. The other three experiments were conducted in a greenhouse at Purdue University (March–April 2000; October–November 2000; and March–April 2001). The F_1 hybrid of Ning 894037/Alondra was also evaluated in the last experiment in the greenhouse (March–April 2001). On average, nine plants of each RIL and 18 plants of the parent lines were evaluated for disease response in each experiment. The experiments were arranged as a completely randomized design. Individual plants within lines were treated as replications.

Conidia spores of a local isolate of *F. graminearum* were provided by Dr. Gregory Shaner, Department of Botany and Plant Pathology, Purdue University. In all experiments in the field and greenhouse, 10 µl of inoculum, containing 500–1,000 spores, were injected into a floret of the third or fourth spikelet from the tip of the spikes at anthesis. In the 2000 spring greenhouse and the 2000 field evaluation, the inoculated spikes were covered with plastic bags for 3 days to maintain high humidity. In subsequent greenhouse tests, inoculated plants were placed under a fine mist for 4 days. Plants were misted for 3 min of every 30 min. Disease symptoms were scored at 15 and 20 days after inoculation in the greenhouse but only once (at 25 days) in the field. The number of discolored spikelets and the total number of spikelets were recorded. Only the spikelets below and including the inoculation point were considered diseased. Disease severity was calculated as the (number of diseased spikelets/total number of spikelets) $\times$ 100%.

DNA isolation and bulked segregant analysis

Ten to 12 seeds of each line in the $F_{8:9}$ generation were germinated for DNA extraction. Genomic DNA was isolated from the seedling leaves using the cTAB method described by Saghai-Maroof et al. (1984). DNA concentration was quantified on a Hoefer DyNA Quant 200. DNAs of the 15 most-resistant RILs, based on the average of the four tests, were pooled in equal quantities to construct a resistant (R) bulk. Likewise, DNAs from the 15 most-susceptible RILs were pooled to create the susceptible (S) bulk. The two bulks and the population parents were screened for polymorphism with 251 SSR markers, 186 from Röder et al. (1998) and 65 from Cregan and coworkers (unpublished). Once polymorphisms were identified between the two bulks, individuals of the two bulks were screened for that marker. A one-way analysis of variance was performed to test significance of the different disease severity between the two marker classes with the 30 lines in the R and S bulks. If it was significant at $\alpha = 0.01$, the whole population was genotyped for the marker. Additional polymorphic SSR markers known to be closely linked to those significant markers were also characterized to establish regional linkage maps.

PCR amplification was conducted in a 25-µl reaction mix, which contained 1 $\times$ buffer (Promega), 1.5 mM of $MgCl_2$, 2.0 mM of dNTPs, 250 µM of oligonucleotide primers, 40 ng of DNA, and 1 unit of *Taq* polymerase. PCR was performed in an MJ Research Thermal Cycler (PTC-100 programable Thermal Controller). The samples were denatured at 94 °C for 2 min, followed by 35 cycles consisting of 94 °C for 30 s, 55 °C (or 60 °C) for 40 s, 72 °C for 1 min, with a final extention at 72 °C for 7 min. The choice of 55 °C or 60 °C for annealing is according to the information provided for the primers (Röder et al. 1998; Cregan et al., personal communication). The amplified products were separated on a 3% Metaphor agarose gel containing ethidium bromide and visualized with a UV transilluminator.

Statistical analysis

Analyses of variance for disease severity in each experiment and a combined analysis across the four experiments were performed using the "PROC GLM" procedure of the SAS software package (SAS Institute Inc., Version 8.0, 1999). Broad-sense heritability was calculated based on variance components calculated using SAS "PROC VARCOMP". Both RILs and experiments were considered as random effects. The variance component for RILs (σ_g^2) was treated as genetic variance. Variance components for experi-

Table 1 Mean and standard deviation of disease severity (percentage of discolored spikelets per spike) in Ning 894037, Alondra, F_1 hybrid and RILs from the cross of the above two cultivars

Genotype	Disease severity (%)				
	00 SGH[a]	00 Field	00 FGH	01 SGH	Combined
Ning 894037	4.4 ± 1.7	19.1 ± 14.1	11.1 ± 7.2	9.3 ± 6.0	13.7 ± 15.8
Alondra	30.9 ± 20.3	60.2 ± 27.5	63.6 ± 37.9	61.3 ± 38.6	53.0 ± 34.0
F_1	–[b]	–	–	34.6 ± 31.6	34.6 ± 31.6
RILs	21.6 ± 18.4	40.1 ± 23.8	35.5 ± 20.2	44.3 ± 28.4	35.6 ± 18.8
Minimum	3.2 ± 0.2	5.6 ± 2.0	5.6 ± 2.1	4.4 ± 1.2	6.6 ± 5.4
Maximum	88.5 ± 20.3	100 ± 0	88.5 ± 19.0	100 ± 0	84.3 ± 24.2
LSD.05	21.4	25.7	30.0	29.3	12.7

[a] 2000 spring greenhouse; 2000 field; 2000 fall greenhouse; 2001 spring greenhouse
[b] F_1 was not evaluated in 00 SGH, 00 Field, and 01 SGH

ments (σ_e^2), RILs × experiment interaction ($\sigma_{g\times e}^2$) and error (σ^2) were combined to estimate environmental variance. The equation used to describe heritability is:

$$H^2 = \sigma_g^2/(\sigma_g^2 + \sigma_{g\times e}^2 + \sigma_e^2 + \sigma^2) \text{ based on plot mean, and}$$

$$H^2 = \sigma_g^2/(\sigma_g^2 + \sigma_{g\times e}^2/r + \sigma_e^2/rn) \text{ based on entry mean,}$$

where r is the number of experiments, and n is the average number of plants per line (replications) evaluated in each experiment.

Correlation coefficients among pairs of experiments were calculated with the "PROC CORR" procedure. Marker order was established with MapMaker/Exp 3.0b (Lander et al. 1987) with the "compare" command (LOD threshold 3.0). A Kosambi map function was applied to calculate the distance between ordered markers. Composite interval mapping (Zeng 1994) was conducted with the software package QTL Cartographer to detect the association of SSR markers and a QTL. Five markers and a 10-cM window size were used as a background control. The threshold of the LOD score for suggesting the significance of a QTL was determined by a 1,000-permutation test (Doerge and Churchill 1996). Multiple regression of the three QTLs on the phenotype was performed with the "PROC REG" procedure of SAS.

Results

Phenotypic variation

Data based on the first reading of disease-spread were skewed toward resistance in the frequency distribution in all three experiments in the greenhouse. The second reading was normally distributed except in the March–April 2000 greenhouse test. Thus, only the data recorded at 20 days were used in the analyses.

Mean disease severity of the two parents differed significantly in each of the four experiments (Table 1). In the 2001 spring greenhouse experiment, disease severity of the F_1 hybrid was 34.6%, while disease severity of the two parents was 9.3% and 61.3%, respectively. Pairwise comparison among Ning 894037, Alondra and the F_1 showed that the F_1 was significantly different from the parents, but not significantly different from the mid-parent value (LSD = 23.3%, $P = 0.003$), indicating that FHB resistance in this population fits an additive genetic model.

The mean disease severity of RILs averaged over the four experiments ranged from 6.6% to 84.3% and displayed a bell-shaped distribution (Fig. 1), showing large phenotypic variation in the population. Analysis of variance (Table 2) showed significant variation among RILs,

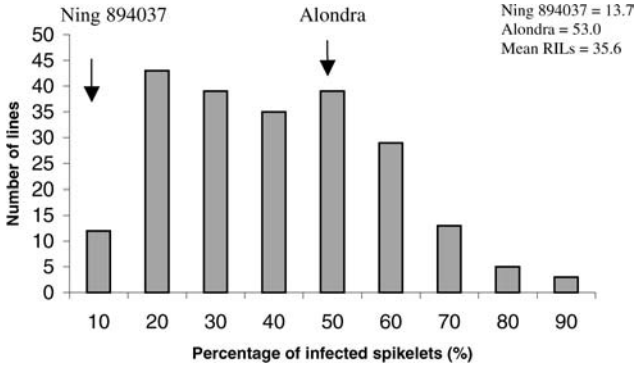

Fig. 1 Distribution of disease severity in the RILs developed from the cross of Ning 894037 and Alondra. Data based on the average of four experiments

Table 2 Analysis of variance for FHB severity across four experiments in the RILs from the cross of Ning 894037 and Alondra

Variables	*df*	Mean square	*F*-value	*P*-value
RILs	217	10,682	14.78	<0.0001
Experiment	3	142,890	197.72	<0.0001
RILs × experiment	649	2,001	2.77	<0.0001
Error	6,796	722		

which represents genetic variation. RILs × experiment interaction was also significant. Broad-sense heritability was estimated to be 0.23 based on plot means and 0.87 based on entry means.

The correlation among the four experiments ranged from 0.47 to 0.61. This was intermediate but highly significantly correlated ($P < 0.0001$). The most resistant lines and susceptible lines correlated more highly than the intermediate lines. For the two extremes, the 15 most-resistant and 15 most-susceptible RILs, the correlation between each of the two experiments ranged from 0.82 to 0.91. These 15 lines of the two extremes were used to construct the two bulks for marker analyses.

SSR marker analysis and QTL mapping

Ninety nine of the 251 SSR primer pairs revealed polymorphism between the two parents. Sixteen of those also

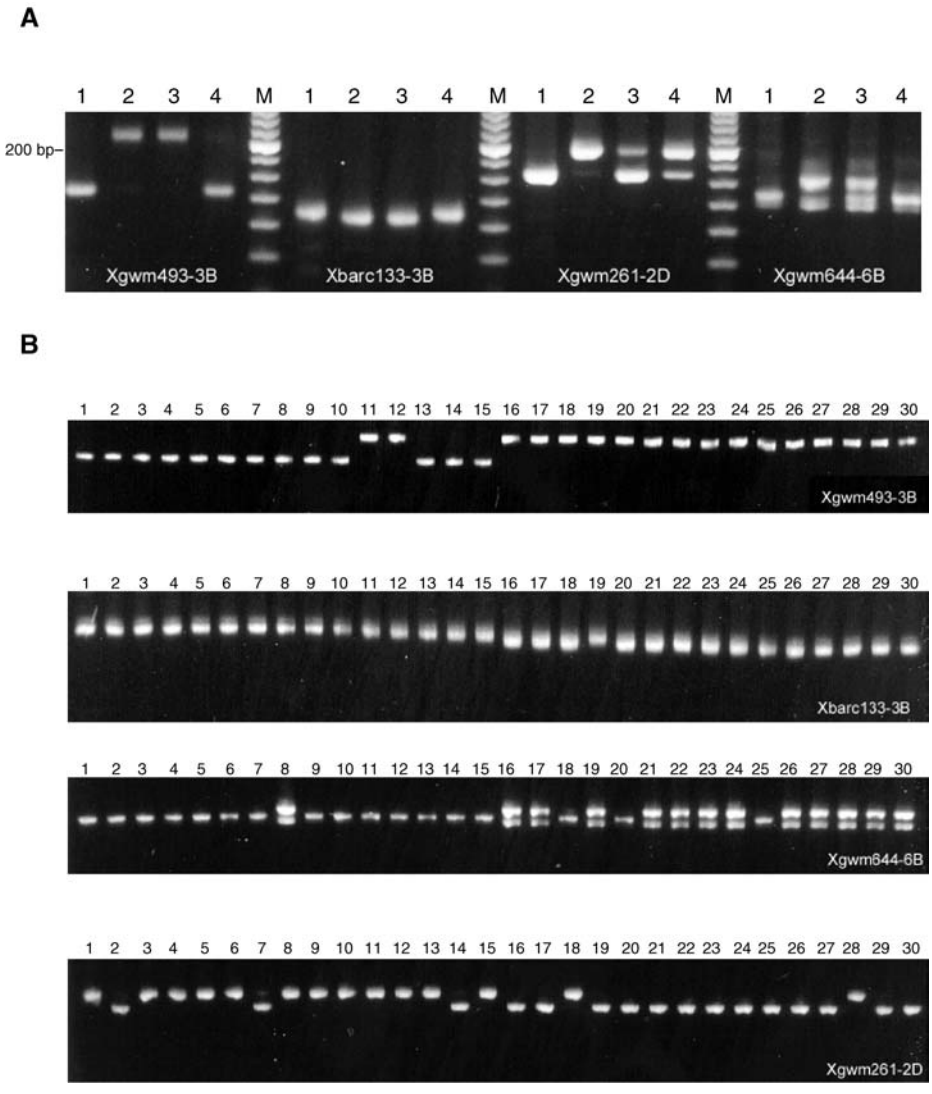

Fig. 2 **A** Gel electrophoresis of the amplification products using SSR markers. *1* Alondra; *2* Ning 894037; *3* Resistant bulk; *4* Susceptible bulk; *M* DNA molecular-weight standard 20-bp ruler. **B** Segregation of markers. *Lanes 1–15* are the 15 susceptible lines in the S-bulk. *Lanes 16–30* are the 15 resistant lines in the R-bulk

Table 3 Summary of the effects of QTLs detected in the Ning 894037/Alondra RI population resulted from composite interval mapping

Interval	Chrom.[a]	Interval length	LOD score[b] ($R^2 \times 100\%$)				
			Exp.1	Exp.2	Exp.3	Exp.4	Combined
Xbarc133-Xgwm493	3BS	10.6 cM	9.0 (19.2%)	19.7 (40.6%)	14.0 (29.5%)	15.3 (29.8%)	24.9 (42.5%)
Xgwm296-Xgwm261	2DS	8.3 cM	5.6 (11.9%)	3.7 (6.1%)	2.9 (5.0%)	7.6 (11.7%)	7.7 (12.1%)
Xgwm88-Xgwm644	6BS	6.2 cM	–[c]	2.3 (3.4%)	1.8 (3.1%)	2.1 (2.9%)	3.1 (4.4%)

[a] Chromosome location
[b] Threshold of LOD score from the 1,000-permutation test is: 1.69 for $P < 0.1$, 2.03 for $P < 0.05$; 2.78 for $P < 0.01$
[c] Not detected in Exp. 1

revealed polymorphism between the bulks (Fig. 2A). The 30 individuals in the two bulks were further genotyped for those 16 SSR markers, and the polymorphism was also evident, with a few recombinations, or mischaracterization of the phenotype (Fig. 2B). An analysis of variance was performed to test the significance of marker-trait association for each marker in all RILs in the two bulks. All the 16 markers were significant at $\alpha = 0.01$. Thus, they were screened in the whole population. Using general linear regression, we detected 13 markers significantly associated with FHB resistance at $\alpha = 0.05$ in the population. They were assigned to three linkage groups by the software MapMaker/EXP 3.0b. According to Röder et al. (1998) and Ward's (personal communication) genetic linkage maps, they are located on wheat chromosomes 3B, 2D and 6B, in the order of magnitude of their effects. With additional SSR markers closely linked to these markers, we established a regional linkage map and

Table 4 Multiple regression estimates of phenotypic effects of the three QTLs on the disease severity in the RI population of Ning 894037/Alondra ($R^2 = 0.516$)

Interval	Chromosome	Effects[a]	SE	*t*-value	$P > \|t\|$
Xbarc133-Xgwm493	3BS	26.6	2.6	10.3	<0.0001
Xgwm261-Xgwm296	2DS	–12.5	2.8	–4.5	<0.0001
Xgwm644-Xgwm88	6BS	9.4	2.7	2.35	0.0007

[a] Regression coefficients in the multiple regression model. Positive and negative numbers indicate contributions from Ning 894037 and Alondra, respectively

conducted a composite interval mapping with QTL Cartographer. The output of the analysis also inferred that there were QTLs in these three genomic regions. The effect of the QTL on 3BS was significant in all four experiments with a high LOD score (9.0, 19.7, 14.0 and 15.3, respectively). The percentage of phenotypic variation that can be explained by this QTL ranged from 19.2% to 40.0% in individual experiments (Table 3). With combined data, R^2 was as high as 0.425. A linkage map around this region (3BS) was constructed with nine SSR markers spanning 125 cM. The location of the QTL was at the interval of Xbarc133 and Xgwm493, which are 10.6-cM apart (Fig. 3). The moderately susceptible parent Alondra contributed a resistance QTL on 2DS, located at the interval of Xgwm296 and Xgwm261. This QTL was detected in all four experiments. The LOD score ranged from 2.8 to 7.6; R^2 ranged from 0.05 to 0.121. The third QTL, on 6BS, has a relatively small effect. It was detected in three experiments as well as the combined data. The R^2 ranged from 0.029 to 0.044 (Table 3). A linkage map consisting of six SSR markers on 6B was also established, which spans 53.6 cM (Fig. 3).

Multiple linear regression

The three closest markers, Xbarc133(3BS), Xgwm261(2DS) and Xgwm644(6BS), jointly explained 36.3% of the phenotypic variation (data not shown). If both of the flanking markers were used, the R^2 increased to 0.516, which means that these three intervals account for 51.6% of the phenotypic variation. Multiple regression analysis showed that the QTL associated with the interval between Xbarc133 and Xgwm493 on 3BS decreases disease severity by 26.6%. The other two QTLs, on chromosomes 2D and 6B, decrease severity by 12.5% and 9.4%, respectively (Table 4).

The origin of the 3BS QTL

SSR analyses using the flanking markers of the 3BS QTL (Xgwm533, Xgwm493) showed that the three USA sources of Funo, CItr 14349, PI 213833, PI 303665, amplified identical DNA bands, but they are not the same bands that Sumai 3 has. However, data showed that Sumai 3 inherited the two markers from its other parent Taiwan Wheat. Ning 894037 has the same resistant bands as Sumai 3 for both markers. Sumai 3 and Taiwan Wheat have an additional band, the Xgwm533.2 locus, whereas Ning 894037 does not have it (Fig 4).

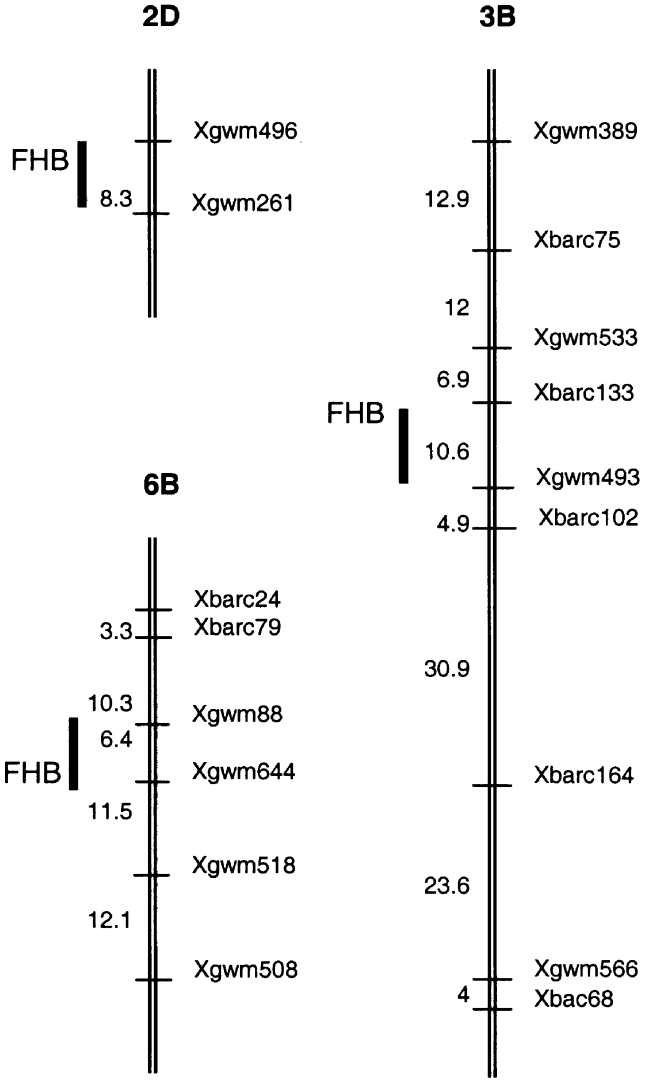

Fig. 3 Genetic map indicating the position of three FHB resistance QTLs relative to SSR loci. *Numbers* at the interval of each marker represent the distance in cM

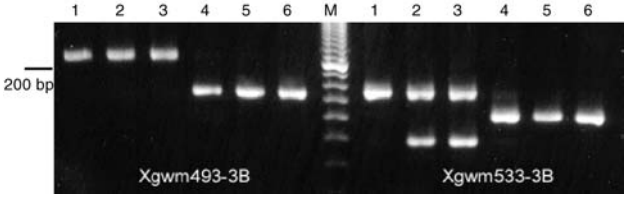

Fig. 4 SSR analysis showing that the origin of the 3BS QTL in Sumai 3 is from Taiwan Wheat, and Ning 894037 has the same resistant bands as Sumai 3. *1* Ning 894037; *2* Taiwan Wheat; *3* Sumai 3; *4* CItr 14349; *5* PI 213833; *6* PI 303665

Discussion

Evaluation of a recombinant inbred population from the cross, Ning 894037/Alondra, for Fusarium head blight resistance suggests that the resistance is a quantitative trait. QTL mapping indicates that the QTL with largest effect is located on the short arm of chromosome 3B in the interval flanked by SSR loci Xbarc133 and Xgwm493. This interval or QTL is contributed by Ning 894037, and it explained 42.5% of the phenotypic variation for FHB resistance. A similar QTL position was reported by Waldron et al. (1999), Anderson et al. (2001), Buerstmayr et al. (2002) and other researchers. With no exception, their resistance sources were Sumai 3 or its derivatives. We conclude that there really is a QTL with large effect on chromosome 3BS, and the QTL of Ning 894037 most likely is the same as that of Sumai 3, because the flanking markers are the same in Ning 894037 and Sumai 3. As we knew previously, Ning 894037 did not have Sumai 3 as a parent, but the Italian line Funo was their common parent. Sumai 3 is derived from the cross of Funo/Taiwan Wheat. Yangmai 3, the somaclonal parent of Ning 894037, was a selection for earliness from Funo. Thus, from the pedigree of Ning 894037, it was believed that the resistance to FHB came from the Italian cultivar Funo. However, our study showed that Funo does not have either one of the flanking markers for this QTL on 3BS, and that both Ning 894037 and Sumai 3 do. Instead, Sumai 3 inherited the 3BS chromosome region from Taiwan Wheat (Fig. 4). Thus, we conclude that the FHB resistance QTL on 3BS is from the Chinese line, Taiwan Wheat. AFLP fingerprinting also suggested that Sumai 3's QTL on 3BS came from Taiwan Wheat (Bai et al. 2001). Where did the 3BS QTL of Ning 894037 come from? In the lineage from Funo to Yangmai 3 to Ning 894037 there was no other cultivar involved in the development of Ning 894037. A possible explanation is that the FHB resistance QTL of Ning 894037 on 3BS came from outcrossing. As we know, the male fertility of regenerated plants from tissue culture is usually low, increasing the probability of outcrossing.

The QTL on chromosome 2D predicted 12.1% of the phenotypic variation for FHB resistance. This QTL is contributed by the moderately susceptible parent, Alondra. Alondra was typically considered susceptible when inoculated at the middle of the spikes. All spikelets of Alondra above the inoculation point became wilted. Considering that the wilted spikelets may be caused by the limited water and nutrients rather than infection by Fusarium, as revealed by the bioassay of floral components following inoculation (Cheryl et al. 2001), we inoculated the flower of the third or fourth spikelet from the tip of the spikes. Only those spikelets below and including the inoculation point were considered diseased. In this way, Alondra showed some resistance compared to other more-susceptible cultivars such as 'Clark'. Typically in our tests, only one-half to three-fourths of the spikelets of Alondra became diseased. It appeared that counting the infected spikelets below the inoculation point is a more useful evaluation than counting the total of wilted and diseased spikelets. A QTL of similar chromosomal location was reported by Xu et al. (2001), with a combination of SSR and AFLP markers in a doubled-haploid population derived from the cross of Sumai 3/'Gamenya'. Similar to our results, the 2D QTL in their population was also contributed by the more-susceptible parent, Gamenya. In an early genetic study of Sumai 3's FHB resistance, Yao et al. (1997) constructed 21 substitution lines. With the variety Chinese Spring as the genetic background, each chromosome of Chinese Spring was replaced by a corresponding chromosome of Sumai 3. They found that the 2D substitution line was more susceptible than Chinese Spring. In other words, Chinese Spring may possess resistance on 2D. Their results support our conclusion that there is a FHB resistance QTL on chromosome 2D.

We used bulked segregant analysis (BSA) to detect the QTLs conditioning FHB resistance. This strategy is efficient for gene mapping because it allows a considerable research-saving compared to comprehensive genotyping. BSA is generally not regarded as a useful approach for either detection of QTLs for quantitative traits which may be conditioned by several genes with small effect, or when the QTL is loosely linked to the marker. This is because the two bulks are frequently contaminated with alternative alleles if mischaracterization exists or recombination occurs (Wang and Paterson 1994). However, this study demonstrates that with a large population and precise phenotypic characterization, it is possible to detect QTLs with small effects. It is true that the two bulks were contaminated by alternative alleles, as with the marker Xgwm261. The polymorphism revealed by the marker Xgwm261 between the two bulks is the difference in allelic frequency. That is, both alleles appear in each bulk but the difference in intensity of each allele is obvious, indicating the relative difference in allele frequencies. SSR assays with this marker in the individuals of the two bulks showed that three out of the 15 susceptible lines had the resistant band, and two out of the 15 resistant lines had the susceptible band (Fig. 2). In our study, the QTL on 6B was relatively small, explaining 3–4% of the phenotypic variation, but we were still able to detect it in three of the four experiments, as well as with the combined analysis over the four experiments. The QTL was not detected in the March–April 2000 greenhouse test, probably because of the retarded disease progress in that test.

With multiple regression of the three QTLs on disease severity, these three QTLs accounted for 51.6% of the phenotypic variation in our population, and predicted 59% of the genetic variance. Thus, it is possible that additional QTLs remain undetected. There may be two reasons that account for our failure to detect additional QTLs in this population: the low coverage of SSR markers on the 21 wheat chromosomes, and the ineffectiveness of BSA methodology to detect epistasis.

In recent years gene-mapping research led to the discovery of the QTL with a large effect on 3BS and several QTLs with smaller effects (Waldron et al. 1999; Zhou et al. 2000; Anderson et al. 2001; Buerstmayr et al. 2002). Transgressive segregation, revealed either by classical quantitative analysis or molecular mapping, is not uncommon (Snijders 1990b; Singh et al. 1995; Waldron et al. 1999). The major gene on 3B alone appears not sufficient to prevent severe epidemics under field conditions. Taiwan Wheat, the donor of the 3BS QTL to Sumai 3, is a moderately susceptible cultivar (Bai and Shaner 1994). Sumai 3 is a result of transgressive segregation from Taiwan Wheat and Funo. Also, FHB resistance in wheat involves complex interactions with the environment. Although broad-sense heritability is high (0.87) based on entry means, we should note that this high heritability is achieved as a result of a large number of plants being tested across multiple environments, with a recombinant inbred population. Heritability based on plot means is as low as 0.23, due to a large error variance, variance of the environment, and variance of G × E interaction. Similar results were reported by Waldron et al. (1999) and Anderson et al. (2001). In a breeding program, it is not practical to screen a large number of lines in such a way. The utilization of markers closely linked to target genes for gene pyramiding may overcome this problem of low heritability. In this study, we identified a SSR marker (Xbarc133) closer to the 3BS QTL than previous reports. This would be useful for marker-assisted selection.

Acknowledgements This research was supported in part by the US Wheat and Barley Scab Initiative, USDA/ARS, Award No. 59-0790-9-057 to H. Ohm; and by the Hi-tech research and development program of China, project No. BH02-03-01 and EU-INCO, project No. ERBIC18CT98 0312 to W. Lu. We thank Marion S. Röder [Institut für Pflanzengenetik und Kulturpflanzenforschung (IPK), 06466 Gatersleben, Germany] and Perry Cregan (Soybean Genomics and Improvement Laboratory, USDA, ARS, Beltsville Agriculture Research Center, Beltsville, Md. 20705) for making SSR primer sequences available; and Richard Ward (Department of Crop and Soil Science, Michigan State University, East Lansing, Mich. 48824) for mapping information for the BARC SSR markers.

References

Anderson JA, Stack RW, Liu S, Waldron BL, Fjeld AD, Coyne C, Moreno-Sevilla B, Mitchell Fetch J, Song QJ, Cregan PB, Frohberg RC (2001) DNA markers for Fusarium head blight resistance QTLs in two wheat populations. Theor Appl Genet 102:1164–1168

Bai GH, Shaner G (1994) Scab of wheat: prospects for control. Plant Dis 78:760–766

Bai GH, Guo P, Kolb FL (2001) Genetic diversity in scab-resistant wheat cultivars based on molecular markers (Abstract). In: National Fusarium Head Blight Forum Proceedings, 8–10 December, Cincinnati, p 7

Buerstmayr H, Lemmens M, Hartl L, Doldi L, Steiner B, Stierschneider M, Ruckenbauer P (2002) Molecular mapping of QTLs for Fusarium head blight resistance in spring wheat. I. Resistance to fungal spread (Type II resistance). Theor Appl Genet 104:84–91

Cheryl EE, Argyris J, Rucker M, Serrano H, Tekrony DM (2001) *Fusarium graminearum* infection and movement in floral components of wheat spikes (Abstract). In: National Fusarium Head Blight Forum Proceedings, 8–10 December, Cincinnati, p 115

Doerge RW, Churchill GA (1996) Permutation tests for multiple loci affecting a quantitative character. Genetics 142:285–294

Lander ES, Green P, Abrahamson J, Barlow A, Daley MJ, Lincoln SE, Newburg L (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

Lu WZ, Zhou MP, Zhang X (2000) Studies and improvement of wheat breeding for scab resistance using biotechnology. In: Proc Int Symposium on Wheat Improvement for Scab Resistance, 5–11 May, Suzhou and Nanjing, China, pp 151–156

Lu WZ, Chen SH, Wang YZ (2001) Research on wheat scab. Scientific Publishing house, Beijing, China

Mesterhazy A (1995) Types and components of resistance to Fusarium head blight of wheat. Plant Breed 114:377–386

McMullen MP, Jones R, Gallenberg D (1997) Scab of wheat and barley: a re-emerging disease of devastating impact. Plant Dis 81:1340–1348

Miller JD, Young JC, Sampson DR (1985) Deoxynivalenol and Fusarium head blight resistance in spring cereals. Phytopathol Z 113:359–367

Röder MS, Korzun V, Wendehake K, Plaschke J, Tixier MH, Leroy P, Ganal MW (1998) A microsatellite map of wheat. Genetics 149:2007–2023

Saghai-Maroof MA, Soliman K, Jorgensen RA, Allard RW (1984) Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. Proc Natl Acad Sci USA 81:8014–8018

SAS Institute Inc (1999) SAS/STAT user's guide, version 8. Cary, North Carolina

Schroeder HW, Christensen JJ (1963) Factors affecting resistance of wheat to scab caused by *Gibberella zeae*. Phytopathology 53:831–838

Shen XR, Zhou MP, Ren LJ, Huang YH, Zhang JJ, Lu WZ (1998) RFLP analysis of wheat varieties resistant or susceptible to scab. Jiangsu J Agric Sci 14: 129–134

Singh RP, Ma H, Rajaram S (1995) Genetic analysis of resistance to scab in spring wheat cultivar Frontana. Plant Dis 79:238–240

Snijders CHA (1990a) Fusarium head blight and mycotoxins contamination of wheat, a review. Netherland J Plant Pathol 96:187–198

Snijders CHA (1990b) Response to selection in F2 generation of winter wheat for resistance to head blight caused by *Fusarium culmorum*. Euphytica 50:163–169

Van Ginkel M, Van Der Schaar W, Zhuping Y, Rajaram S (1996) Inheritance of resistance to scab in two wheat cultivars from Brazil and China. Plant Dis 80:863–867

Waldron BL, Moreno-Sevilla B, Anderson JA, Stack RW, Frohberg RC (1999) RFLP mapping of QTL for Fusarium head blight resistance in wheat. Crop Sci 39:805–811

Wang GL, Paterson AH (1994) Assessment of DNA pooling strategies for mapping of QTLs. Theor Appl Genet 88:355–361

Xu DH, Juan HF, Nohda M, Ban T (2001) QTLs mapping of Type I and Type II resistance to FHB in wheat. In: National Fusarium Head Blight Forum Proceedings, 8–10 December, Cincinnati, pp 40–42

Yao JB, Ge YF, Wang SW (1997) Chromosomal location of genes for scab resistance in wheat cultivar Sumai 3. Acta Agron Sinica 23:450–453

Zeng ZB (1994) The precision mapping of quantitative trait loci. Genetics 136:1457–1468

Zhou WC, Kolb FL, Bai GH, Shaner G, Domier LL (2000) SSR mapping and sub-arm physical locating of a major scab resistance QTL in wheat. In: National Fusarium Head Blight Forum Proceedings, 10–12 December, Cincinnati, pp 69–72