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DNA markers for Fusarium head blight resistance QTLs in two wheat populations

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Abstract Genetic resistance to Fusarium head blight (FHB), caused by *Fusarium graminearum*, is necessary to reduce the wheat grain yield and quality losses caused by this disease. Development of resistant cultivars has been slowed by poorly adapted and incomplete resistance sources and confounding environmental effects that make screening of germplasm difficult. DNA markers for FHB resistance QTLs have been identified and may be used to speed the introgression of resistance genes into adapted germplasm. This study was conducted to identify and map additional DNA markers linked to genes controlling FHB resistance in two spring wheat recombinant inbred populations, both segregating for genes from the widely used resistance source 'Sumai 3'. The first population was from the cross of Sumai 3/Stoa in which we previously identified five resistance QTLs. The second population was from the cross of ND2603 (Sumai 3/Wheaton) (resistant)/ Butte 86 (moderately susceptible). Both populations were evaluated for reaction to inoculation with *F. graminearum* in two greenhouse experiments. A combination of 521 RFLP, AFLP, and SSR markers were mapped in the Sumai 3/Stoa population and all DNA markers associated with resistance were screened on the ND2603/Butte 86 population. Two new QTL on chromosomes 3AL and 6AS wer found in the ND2603/Butte 86 population, and AFLP and SSR markers were identified that explained a greater portion of the phenotypic variation compared to the previous RFLP markers. Both of the Sumai 3-derived QTL regions (on chromosomes 3BS, and 6BS) from the Sumai 3/Stoa population were associated with FHB resistance in the ND2603/Butte 86 population. Markers in the 3BS QTL region (*Qfhs.ndsu-3BS*) alone explain 41.6 and 24.8% of the resistance to FHB in the Sumai 3/Stoa and ND2603/Butte 86 populations, respectively. This region contains a major QTL for resistance to FHB and should be useful in marker-assisted selection.

Keywords Wheat · Scab · QTL mapping · Disease resistance · *Fusarium graminearum*

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Introduction

Fusarium head blight, caused by *Fusarium graminearum* Schwabe [telomorph: *Gibberella zeae* Schw.(Petch)], is recognized as one of the most-destructive diseases of wheat. Due to quantitative inheritance and difficulties in screening for the presence of resistance genes (Bai and Shaner 1994), DNA markers associated with such genes may increase the efficiency of selecting for resistance.

Previously, from analysis of a recombinant inbred population from the cross 'Sumai 3'/'Stoa', we identified DNA markers for a major FHB resistance QTL on chromosome 3BS (Waldron et al. 1999). This QTL, designated

Qfhs.ndsu-3BS, was derived from the Chinese cultivar Sumai 3, a widely used resistance source for this disease. Four other QTLs associated with resistance were identified, two of which were derived from Sumai 3. Bai et al. (1999) identified a major QTL for FHB resistance in 'Ning7840', which is believed to have inherited its scab resistance from Sumai 3 (Liu and Wang 1990). Contrary to the original tentative location of this QTL on chromosome 7B (Bai et al. 1999), this gene is believed to reside in the *Qfhs.ndsu-3BS* region (Bai, personal communication).

Exploitation of marker-QTL linkages in breeding programs requires robust markers that are effective across genetic backgrounds. A further requirement is that such diagnostic approaches provide efficiencies compared with conventional, phenotype-based procedures. There are few documented cases of using DNA markers for QTLs in applied plant breeding programs (Young 1999). A contributing factor to this failure is that the QTLs often show G×E effects, or their effects in new genetic backgrounds are less pronounced (Tanksley and Nelson 1996).

The objectives of the present research were to verify the FHB QTLs identified in the Sumai 3/Stoa population with another population and obtain more closely linked markers to *Qfhs.ndsu-3BS*.

Materials and methods

FHB screening

A population of 112 F5-derived recombinant inbred lines (RIL) from the cross Sumai 3 (resistant)/Stoa (mod. susceptible) grown in the greenhouse was evaluated for reaction to inoculation with conidia from *F. graminearum* in two experiments, as described by Waldron et al. (1999). A population of 139 F5-derived recombinant inbred lines (RIL) from the cross ND2603 (Sumai 3/Wheaton) (resistant)/Butte 86 (moderately susceptible) was evaluated for reaction to inoculation with conidia from *F. graminearum* in two greenhouse experiments, as described by Mitchell Fetch et al. (1998). Briefly, at anthesis, an average of nine spikes of the same size and maturity in each of three replications per RIL were inoculated with a 10-µl droplet (50,000 conidia/ml) of conidial suspension placed directly into a single spikelet near the center of the spike, following procedures described by Stack (1989). The conidial suspension contained a mixture of three *F. graminearum* isolates in all experiments except for the first Sumai 3/Stoa which used experiment a single isolate. This procedure bypasses primary infection and targets Type II resistance (Schroeder and Christensen 1963; Wang and Miller 1988). A gentle overhead mist was applied and plants were covered with a plastic humidity tent for three nights following inoculation. Three weeks after inoculation, spikes were scored individually for visual symptoms on a 0–100% FHB severity scale (Stack and McMullen 1985). For both populations, the entry means of the two experiments that included all RI lines were used in subsequent analyses.

DNA marker analysis

RFLP mapping in the Sumai 3/Stoa population was described by Waldron et al. (1999). Only those RFLP markers significantly associated ($P<0.05$) with FHB on the Sumai 3/Stoa population were screened for polymorphism and mapped in the ND2603/Butte 86 population.

Because of difficulties in obtaining linked markers for genomic regions significantly associated with FHB resistance (e.g., 3BS), AFLP analysis was initiated to target markers to these regions using a selective genotyping approach. We selected ten RI lines (five resistant and five susceptible) based on the FHB reaction of lines and/or the RFLP genotype of markers in the target region. We used the AFLP kits from Gibco, BRL with slight modifications from the manufacturer's instructions. Primer pairs revealing segregation that suggested association with resistance or linkage to the intended RFLP marker in the ten RILs composing the selective genotyping array were used to genotype all members of the population. All other polymorphic fragments revealed by such primer pairs were mapped on the entire population. Only one pair of AFLP primers (E40M59) was mapped in the ND2603/Butte 86 population. The AFLP markers are designated according to the nomenclature of McIntosh et al. (1998).

Primers for all microsatellites (SSRs) published by Röder et al. (1998) were synthesized and screened for polymorphism among the four parents of these populations. Markers known to be located in putative QTL regions based on our previous research were given the highest priority. PCR amplification was as described by Röder et al. (1998) except that 35 cycles of amplification were used instead of 45. Visualization of fragments was by electrophoresis in 5% polyacrylamide gels and silver staining according to the protocol of Bassam et al. (1991). Polymorphic markers were mapped in both populations. Fifty microsatellites, designated with the prefix 'BARC', were kindly provided by Q.J. Song and P. Cregan, USDA-ARS, Beltsville, Md. These were screened for polymorphism in the parents and aneuploid stocks to identify those located on chromosome 3BS. All such polymorphic markers on chromosome 3BS were used to screen the populations.

Linkage maps were constructed using MAPMAKER Macintosh v2.0 (Lander et al. 1987) and aneuploid analysis was used to determine the chromosomal arm location of markers in linkage groups as described by Anderson et al. (1992). Markers were subjected to regression and interval analysis using the computer program QGENE (Nelson 1997) to identify significant ($P<0.01$) associations between individual DNA markers and FHB resistance. Multiple regression models were developed by initially including the single best marker in all QTL regions and eliminating those that were not significant at $P<0.01$ in the model one at a time. Two-way epistatic interactions involving one of the significant markers were calculated using all markers mapped in the respective population.

Results and discussion

The FHB data for the Sumai 3/Stoa cross was described in Waldron et al. (1999). The ND2603/Butte 86 population displayed a normal distribution, transgressive segregants, and significant variation among RILs for FHB severity (Table 1, Fig. 1). Heritability on an entry mean and plot basis was 0.50 and 0.24, respectively. A large component (34%) of the phenotypic variance for FHB severity in the ND2603/Butte 86 population was the RI line×experiment interaction (Table 1). This source of variation accounted for only 17% of the variation in the Sumai 3/Stoa population (Waldron et al. 1999). This result emphasizes the importance of multi-experiment data for this trait.

In addition to the 360 RFLP loci mapped by Waldron et al. (1999) in the Sumai 3/Stoa population, we mapped 151 loci resulting from 16 pairs of AFLP primers and ten SSRs. The AFLP primer sets were chosen because each had at least one polymorphic fragment that was suggestive of being associated with FHB resistance based on the selective genotyping array of ten individuals. However, such fragments, when mapped on the entire population, were no more likely to be associated with QTLs than other fragments that segregated independent of FHB reaction in the ten individuals (data not shown). Four genomic regions containing putative quantitative trait loci (QTLs) were as-

Table 1 ANOVA table and proportion of phenotypic variation for Fusarium head blight from recombinant inbred lines from the cross ND2603/Butte 86

Source[a]	*df*	MS	*F*-ratio	*P*	Proportion of phenotypic variance[b]
Experiments	1	35,621	65.22	0.013	
Replications (in experiments)	4	546	2.82	0.025	
RI lines	138	1,313	1.98	<0.001	0.24
RI lines×experiments	138	662	3.41	<0.001	0.34
Error	546	194			0.42
Total	827				

[a] Experiments and Replications were considered to have random effects, and RI lines fixed effects

[b] Heritability was 0.50 and 0.24 on an entry mean and per plot basis, respectively

Table 2 Coefficients of determination and *P* values for DNA markers associated with Fusarium head blight resistance in the Sumai 3/Stoa and ND2603/Butte 86 recombinant inbred populations

Marker[a]	Chromosome	Source of resistance allele[a]	Sumai 3/Stoa		ND2603/Butte 86	
			R^2×100	*P*	R^2×100	*P*
Xgwm493/Xgwm533	3BS	Sumai 3/ND2603	41.6	<0.001	24.8	<0.001
XksuH16	2AL	Stoa	14.3	<0.001	0.0	0.66
XksuH4	6AS	Sumai 3/ND2603	1.0	0.32	11.6	<0.001
XBARC101/Xbcd1383	6BS	Sumai 3/ND2603	9.2	0.001	4.9	0.009
Xwg909	4BS[b]	Stoa	7.2	0.007	0.1	0.28
Xbcd941	3AL	ND2603	0.1	0.48	9.1	<0.001

[a] Markers and resistance sources to the left of the slash refer to the Sumai 3/Stoa population and to the right refer to the ND2603/Butte 86 population

[b] This marker was reported as being located on 4BL by Waldron et al. (1999). Due to the position of new markers mapped in this region, the arm position of this marker is believed to be 4BS

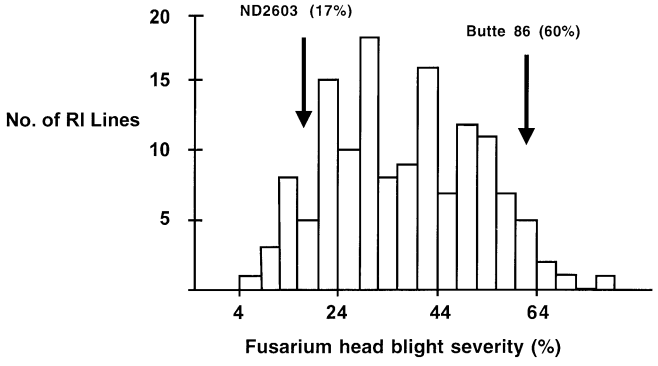

Fig. 1 Histogram of mean percentage of Fusarium head blight severity for parents and 139 RIL lines from the cross ND2603/Butte 86

sociated (P<0.01) with FHB resistance from the combined analysis of two experiments, two from Sumai 3 and two from Stoa (Table 2). The two major QTLs on chromosomes 3BS and 2AL, and a minor QTL on chromosome 4BS, were identified by Waldron et al. (1999). The marker *Xgwm493* is a SSR significantly associated with FHB resistance with an R^2 value of 41.6% (Table 2). This locus mapped in the *Qfhs.ndsu-3BS* region identified by Waldron et al. (1999) and is now the most-strongly associated with FHB resistance in this population. The mapping of AFLPs on chromosome 6BS linked the two minor QTLs reported by Waldron et al. (1999) approximately 24-cM apart. Because neither QTL has a strong or consistent effect in the two environments, we are considering this a single, minor QTL region. A multiple regression model was developed that included all of the markers significantly associated with FHB resistance and listed in Table 2 with the exception of *XBARC101* (Table 3). This model explained 54% of the variation for FHB resistance in this population. Given the heritability on an entry mean basis of 0.78 for FHB resistance in this population (Waldron et al. 1999), we believe that these markers represent all major QTLs segregating in this population. It is likely that other minor QTLs and/or epistatic interactions have not yet been identified.

A total of 62 loci representing all Sumai 3/Stoa QTL regions were mapped in the ND2603/Butte 86 population. Encouragingly, both QTLs associated with FHB resistance from Sumai 3 in the Sumai 3/Stoa population were also associated with resistance in the ND2603/Butte 86 population (Table 2). This is an important step in verifying the effectiveness of these markers in other genetic backgrounds. Two QTL from ND2603, on chromosomes 3AL, and 6AS were also discovered in this population. Neither of the two Stoa-derived QTLs were associated with FHB resistance in the ND2603/Butte 86 population (Table 2). The multiple regression model explained 34% of the variation for FHB resistance in this population and included all of the significant markers listed in Table 2 except *Xbcd1383* (Table 3). No epistatic interactions were found in either population that increased the percent of variation explained by more than 5%. Therefore, these interactions are minor in effect

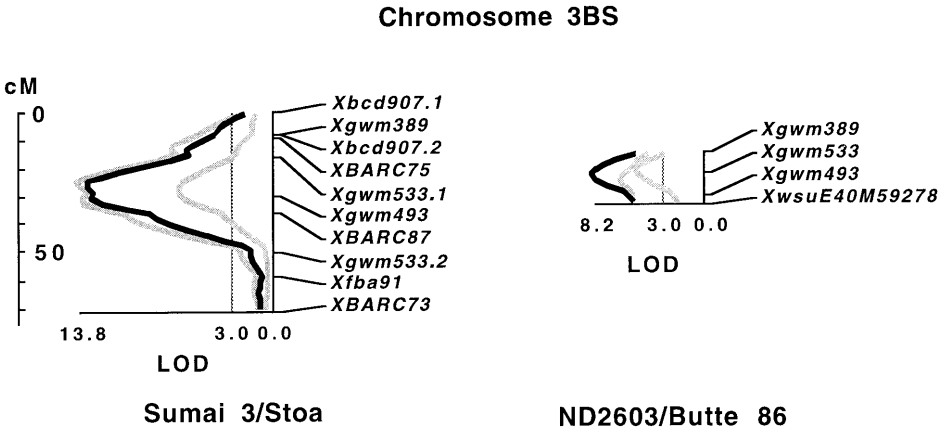

Fig. 2 Interval analysis of data for chromosome 3B for Fusarium head blight resistance in the Sumai 3/Stoa and ND2603/Butte 86 recombinant inbred populations. The *dark contour* in each map represents the mean of the two experiments. The two *lighter colored contours* represent individual experiments. The two maps are aligned at the *Xgwm493* locus. *Xgwm389* and *Xgwm533* were also mapped in both populations

Table 3 Multiple regression models of DNA markers to explain variation in reaction to Fusarium head blight in two spring wheat populations

Source	Estimate[a]	Standard error	*P*
Sumai 3/Stoa[b]			
Xgwm493	–10.32	1.24	<0.001
Xwg909	5.35	1.17	<0.001
XksuH16	4.73	1.24	<0.001
ND2603/Butte 86[c]			
Xgwm533	–5.98	1.11	<0.001
Xbcd941	–3.62	1.08	0.001
XksuH4	–3.60	1.09	0.001

[a] Regression coefficients in the multiple regression models. Positive or negative numbers indicate the direction of the response. Negative numbers indicate lower FHB severity derived from the female parent, either Sumai 3 or ND2603
[b] The coefficient of determination (R^2) was 0.54
[c] The coefficient of determination (R^2) was 0.34

compared to the individual effects of the QTLs identified, and, therefore, were not included in the multiple regression models.

Chromosomes 3B and 6B have been implicated in FHB resistance by monosomic and/or backcross reciprocal monosomic analysis in other wheat germplasms, but not in studies published to-date using Sumai 3 [see Buerstmayr et al. (1999) for a review]. We consider the discovery of QTLs for FHB resistance on chromosomes 3B and 6B using DNA markers in two segregating populations to constitute strong evidence that these represent important genomic regions that in large part are responsible for the resistance observed in Sumai 3. The most significant genomic region associated with FHB resistance in our populations is located on the short arm of chromosome 3B. Interval analysis revealed a peak LOD score of 13.8 and 8.2 for this region in the Sumai 3/Stoa and ND2603/Butte 86 populations, respectively (Fig. 2). The RFLP map of Waldron et al. (1999) gave a peak LOD score of 4.4 for this region. The observation that the multiple QTLs seem to have additive effects, as indicated by the fact that all but one in each population retain significance in multiple regression models, means

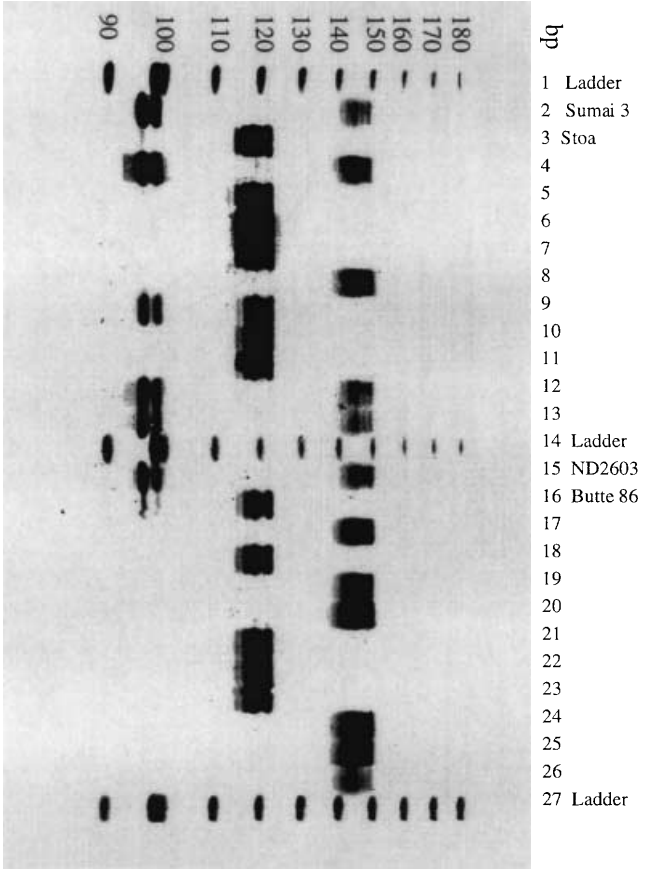

Fig. 3 DNA fragments from amplification of wheat genotypes with the microsatellite marker gwm533. *Lanes 1, 14, and 27* are 10-kb ladder marker lanes; *2, 3, 15, and 16* are parents of the populations; *4–13* and *17–26* are random subsets of progeny from the Sumai 3/Stoa and ND2603/Butte 86 populations, respectively. The fragments centered at 145 and 119 bp are alleles of the *Xgwm533.1* and *Xgwm533* loci in the Sumai 3/Stoa and ND2603/Butte 86 populations, respectively. The fragments between 95 and 100 bp represent the *Xgwm533.2* locus in the Sumai 3/Stoa population. This locus did not segregate in the ND2603/Butte 86 population

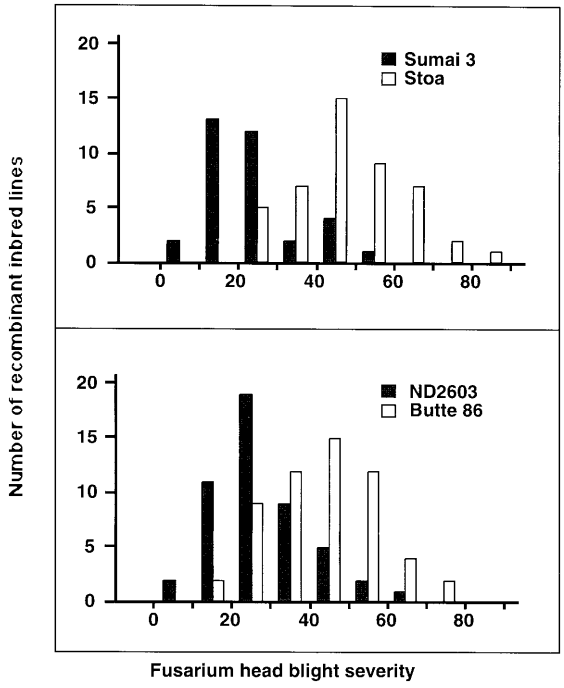

Fig. 4 Histograms of Fusarium head blight severity for RI lines with resistant or susceptible parent alleles in the *Qfhs.ndsu-3BS* QTL region. Only those genotypes homozygous for this interval are included, bound by markers *Xgwm493* and *Xgwm533*

that presumably they can be combined to give greater levels of FHB resistance. However, the effect of *Qfhs.ndsu-3BS* is so large in comparison to the other QTLs (almost twice the effect of the next best QTL in the multiple regression model) that this appears to be the major resistance gene segregating in these populations.

After mapping all of the published SSR markers from chromosome 3BS (Röder et al. 1998) that are polymorphic in both populations, we have positioned *Qfhs.ndsu-3BS* between *Xgwm493* and *Xgwm533* (Fig. 3). Selection for the region bracketed by these two markers greatly skews both populations toward more-resistant types (Fig. 4). This bodes well for the exploitation of these markers as bracketing markers for the QTL which can be used for marker-assisted selection, thereby accelerating the development of resistant cultivars. Future research will include mapping additional markers on 3BS, verifying these and other new markers in additional populations, and initiating marker-assisted selection using markers on 3BS in our wheat breeding program.

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